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THYROID AND GONAD AS FACTORS IN THE PRODUCTION OF PLUMAGE MELANINS IN THE DOMESTIC FOWL.[1]

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I.

In a recent paper, Zavadovsky (1925, 1) has described the production of white feathers on pigmented fowls following thyroid feeding. The dosage was excessive. Some of the birds succumbed; all were highly intoxicated. The survivors soon moulted. Back and wing feathers fell abundantly in the course of ten days, and were succeeded by feathers that were partly or wholly white. Flesh feeding did not produce these effects, nor adrenal gland. The author concluded that "the thyroid plays a specific rôle in the regulation of the growth and moulting of feathers and in their pigmentation."

In the next issue of the same publication, Zavadovsky (1925, 2) described an experiment in which a mongrel black hen under whose skin five dog thyroids had been grafted, developed tufts of white feathers at the seat of the graft. He also observed that "the new plumage which makes its appearance after the experimental moulting is apparently much softer than the old ordinary plumage."

Since 1921, we have had some hundreds of thyroid-fed fowls under observation, and have repeatedly noticed in their plumage occasional feathers with defects in pigmentation similar to those described by Zavadovsky. These have not been regarded as satisfactory evidence of a specific rôle of the thyroid in feather pigmentation. While they could be induced by thyroid feeding,

[1] The work on which this paper is based was done in the Zoölogical Laboratory, University of Oregon.

this was especially true when the dosage was sufficiently large to induce structural defects in the vane as well. Similar defects could be produced by inanition alone. Furthermore, defective pigmentation appeared in birds that were not on a thyroid diet and showed no general signs of hyperthyroidism. When such defects in pigmentation appeared in birds receiving the dosage of thyroid commonly employed by us, namely, 1 gram of Armour and Company's desiccated thyroid to 5,000 grams of body weight, the non-pigmented areas, when they occurred at all, were usually limited to the tips of the feathers involved. This was strictly true for the feathers of the trunk. In wing quills, the extent of the defect was sometimes larger.

With these facts in mind, we are disposed to refer the striking change in plumage recorded by Zavadovsky to a metabolic disturbance induced by an excessive, essentially toxic dosage of thyroid rather than to a specific action of the latter on the pigment-forming mechanism. The results of the graft mentioned in his second paper are not unfavorable to this view. We have not seen the extraordinary casting of feathers in adult birds which we suspect of being a further sequel of toxic feeding. But the moulting process, like the pigment-forming mechanism, is subject to modification under the influence of non-toxic doses of thyroid. This has been referred to in a preliminary note (Horning and Torrey, 1923, 2) and considered more fully in a recent paper (1925, 1). In the latter, two kinds of results of thyroid feeding were discriminated, the one referable to nutritive (associated with toxic), the other to non-nutritive factors. And shortly after (1925, 2), changes in feather structure were described which were not referable to changes in nutrition or to intoxication.

In the present paper, another non-toxic, non-nutritive effect of thyroid feeding will be described, namely, an increased pigmentation. Whereas Zavadovsky's birds *blanched* and not infrequently died under the massive doses pressed upon them, our birds, with a daily ration of thyroid that permitted them to maintain their health, lay viable eggs and rear normal offspring, grew *darker*.

II.

This darkening of the plumage, to which we originally called attention in a preliminary note (1923, 1) was also observed by Cole and Reid (1924). The plate accompanying their paper illustrates very well certain typical color changes produced in the feathers of Brown Leghorns by thyroid feeding. With a view to repeating our observations (1922) on the appearance of plumage of the female type on young Rhode Island Red males as a result of thyroid feeding, these authors found in thyroid fed Brown Leghorn cockerels that had assumed adult plumage changes in feather form and structure that are probably typical responses of poultry in general, but which Crew and Huxley (1923), on a similar errand, failed to note.

What is true of Brown Leghorns in this connection is also true of Campines, Barred Plymouth Rocks and Rhode Island Reds. White Leghorns show similar changes in feather form and structure, though not in color. This exception is likely to prove the rule among other dominant whites as well. There is a definite correlation between structure and color in these experiments, however, which brings both pigmented and non-pigmented races into a single category.

While these different races responded thus similarly to thyroid feeding, they did not do so with equal intensity. Of our pigmented races, the Brown Leghorn was most affected, the Rhode Island Red least. These differences in reaction associated with differences of race, however, were not conspicuous, nor are they so interesting, as those associated with differences in sex.

The results of thyroid feeding are peculiarly conspicuous in Brown Leghorn males, especially in neck hackles, shoulders, backs and saddles, all feathers that are brilliantly colored in shades of red and show marked sexual dimorphism. In the male, these feathers are distinguished by a lacy border of naked barbs (Fig. 1). On a thyroid diet, the latter tend to clothe themselves to their tips with extensions of the normal two rows of barbules (Fig. 2). As the barbules thus extend toward the edge of the feather, so also does the melanin pigment; for, as a rule, this pigment is carried by the barbules and limited by their distribution. As a result of thyroid feeding, the zone of barbules

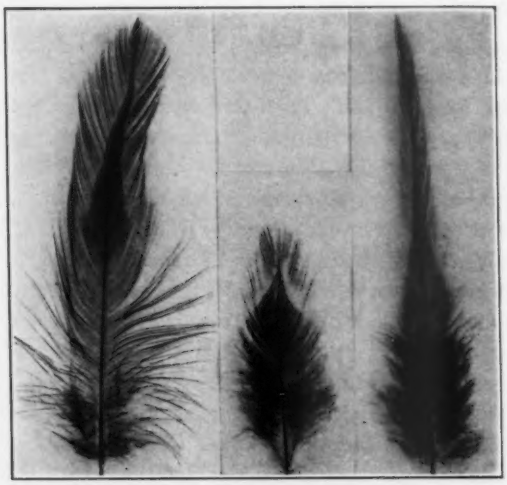

FIG. 1. Representative feathers from a normal adult B. L. male. From left to right they are: hackle, shoulder, saddle.

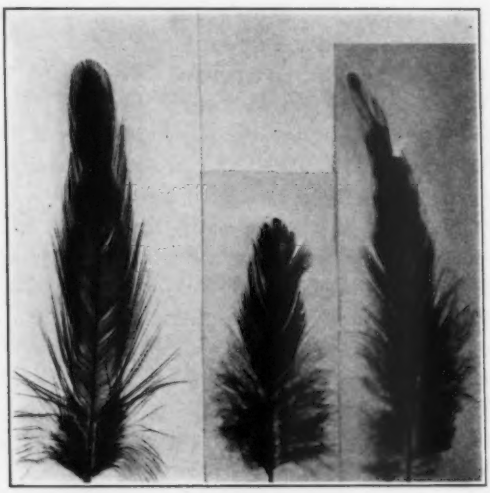

FIG. 2. Representative feathers from a thyroid fed adult B. L. male. From left to right they are: hackle, shoulder, saddle.

may extend beyond the zone of melanin pigmentation; but the reverse does not occur. In control birds, the reverse *does* occur. This is especially clear in the shoulder feathers of Barred Plymouth Rocks, in which dark bands pass distally across the naked barbs (Fig. 3).

FIG. 3. Representative shoulder feathers from an adult Barred P. R. male, showing barring across naked barbs.

This tendency of barbules and melanin pigmentation to extend together over the feathers of thyroid fed birds is especially marked by reason of the fact that the zone of barbules in male birds possesses a typically notched or wavy contour with which the outer edge of melanin pigment distribution perfectly coincides. It is difficult to avoid the conclusion that the effective stimuli for barbule and for melanin formation in these feathers are one and the same. There is reason, however, for believing that this is not an adequate summary of the situation.

Everything that has been said about the male bird applies as well to the capon. In the latter, such decorative feathers

as the hackles, saddles and sickles are longer and possibly more highly colored. The plumage is more luxuriant, *more masculine.* That it becomes more deeply pigmented with melanins as a result of thyroid feeding, however, does not appear from our observations.

In sharp contrast with the effect of thyroid feeding on the coloring of the male bird is its effect on the normal female. Our evidence on this point has been obtained first, from females that had received a daily ration of thyroid approximately 1 : 5000 body weight for at least a year, beginning with the second to fourth week after hatching; second, from females approximately two years old that had been plucked in hackle and saddle regions to stimulate the eruption of new feathers and then placed on the same ration of thyroid as the others.

In the first group, thyroid feeding produced no departure in coloration from the controls. One conspicuous instance of this absence of thyroid effect was supplied by three hens whose parents as well as themselves had been on a thyroid ration practically all their lives. When a year old, they were not distinguishable from their controls.

In the second group were five hens. In the new feathers that appeared on the denuded areas during the period of thyroid feeding there was little or none of the brown mottling characteristic of the controls, and a correspondingly wider distribution of dark pigmentation, less intense than in corresponding feathers of thyroid males, but present in all the experimental birds.

If these observations accurately represent typical conditions, it may be said that the plumage of Brown Leghorn females darkens under the influence of thyroid feeding, as indicated, but not to the same degree as in males of the same age, and apparently not at all in birds in their first year.

A comparable difference in the effect of thyroid feeding on the two sexes has also been observed among Barred Plymouth Rocks. It is the male, rather than the female, whose plumage darkens in the course of the early moults.

These facts suggest a possible inhibitory influence of the ovary in this connection, a view which receives strong support from the following case.

FIG. 4. Thyroid fed B. L. female from which the ovaries have been removed.

FIG. 5. Representative hackles from the bird shown in Fig. 4. arranged from below upward.

A Rose Comb Brown Leghorn female, hatched April 9, 1923, was ovariotomized May 6, 1923. Thyroid feeding was begun at once. Fig. 4 is a photograph of this bird almost two years

FIG. 6. Representative saddle feathers from three adult B. L. females which are, from left to right; the bird shown in Fig. 4, a normal thyroid fed female, and a control.

later, just before she was killed on March 28, 1925. The comb and wattles are large and well formed. The spurs are 2 cm. long. Sickles are present among the feathers of the tail. These are all male characters, assumed as a consequence of ovariotomy. The body conformation is of the female type, as is, in general, the plumage. One exception to this statement has already been noted, namely, the presence of tail sickles. Another is to be found in the neck hackles which, though not well shown in the photograph, approximate in form and structure and color the hackles of thyroid fed male birds. In general, the plumage is duskier than in unaltered females. This is especially true for hackles and saddles. The former may have no lacing, or they may be laced intermittently in the fashion characteristic of thyroid fed males. In neither case, however, do traces of the mottling remain that is so conspicuous in the female hackle. The saddles, which are female in form and structure, show traces of mottling but along with the hackles show an intensity of melanin pigmentation that surpasses the results, mentioned above, on unaltered thyroid fed females.

In post mortem examination of this bird, no trace of a definitive ovary was seen. At the site of the latter, however, were certain small fleshy nodules which revealed, on sectioning, masses of tubules resembling testicular tubules in various stages of development from sex cords, though lacking in definitive sex cells. We have assumed no connection between this tissue and the changes in pigmentation before us. Its significance appears to lie in another direction; and its further consideration will be deferred to another occasion.

While barbule formation and melanin production are both stimulated by thyroid feeding in the male, the presence of the ovary in the thyroid fed female interferes with the second without interfering with the first. Indeed, barbule formation reaches its fullest expression in the female. Lacing, though present in the hackles of the female to a limited extent, is an elaborate and striking feature of those feathers in the male that are especially associated with sex.

The evidence is obviously not sufficient to warrant final conclusions regarding the inhibitory effect of the ovary on pigment formation stimulated by thyroid feeding. It is clear, however, that males and females respond differently to thyroid feeding. And that this difference is due in some measure to the influence of the ovary seems equally true. As for the measure of its influence and the nature of the mechanism by which it is achieved, the matter is not so simple and must await further investigation.

III.

Two classes of feather pigments have been distinguished: *lipochromes*, which are soluble in alcohol and ether and are red, rose brown, yellow and scarlet, and *melanins*, which range from sepia to black. In our experiments, the extension of the latter is correlated with the disappearance of the former. Cole and Reid mention "an evident action toward the reduction of red pigment." We have no evidence of an actual replacement of the one by the other, either by substitution or transformation. It is quite consistent with our observations to assume for the present that the former are merely concealed as the latter advance.

The melanins appear in definite chromatophores, being formed *in situ*. The chromatophores are found in the epidermis of the rachis and barbs. At first they are lacking in pigment which is gradually laid down within them in the form of granules. The pigment of the barbule cells is obtained by a direct transfer from the chromatophores which send out amœboid processes as the barbules develop, these processes meeting and fusing with the barbule cells as though guided by a tropistic factor. Readers who are interested in the details of this remarkable mechanism are referred to the papers of Strong (1902) and Lloyd Jones (1915).

The expansion of melanins in the feathers of thyroid fed birds is conceivably dependent, then, on several factors: an increased amount of melanin pigment; an expansion of individual melanophores, with or without added pigment; an increase in number of melanophores; a migration of melanophores. Considering the large areas ordinarily free of melanins that darken under thyroid feeding, it is difficult to avoid the conclusion that the total amount of melanin is increased. The extension of individual melanophores over very much smaller areas is certainly a fact. Whether or not the melanophores multiply we have not determined. The number of *functional* melanophores assuredly increases. We have no evidence of the migration of melanophores over the long distances required by the observed facts, and it appears to be highly improbable.

The darkening of feathers as a result of thyroid feeding is probably due chiefly, then, to an increase in melanin pigment and an increase in the number of melanophores functioning with or without an increase in the number of cells themselves. Thyroid feeding is thus conceived to promote pigment formation, directly or indirectly, and possibly cell division as well. These are two sufficiently diverse functions, both, in our opinion, indirect. Whether the melanophores do or do not multiply under thyroid influence, there is no doubt that other cells do, namely, the cells of the barbules whose number and extent is so markedly increased. But it is not clear at present in either case through what channels the thyroid acts to augment pigmentation or cell proliferation. Evidence has been given elsewhere (Torrey,

Riddle and Brodie, '25) for the view that thyroxin depresses the division rate when acting directly on Paramecium.

SUMMARY.

The results of thyroid feeding on five breeds of domestic fowl are recorded. They vary with dosage and with sex. When the daily ration is compatible with good health, the males of pigmented breeds, both normal and castrated, tend to *darken* conspicuously owing chiefly to an increased production of plumage melanins. Normal females are affected, if at all, to a much slighter degree. Castrated females, however, resemble males in this respect as in others. *An antagonism thus appears between ovary and thyroid* as indicated by their relation to the formation of plumage melanins. The *blanching* of plumage observed after excessive doses of thyroid is attributed to a toxic effect, non-specific in character. A correlation between the effect of thyroid feeding on melanin production and feather structure is pointed out. White Leghorns show the latter but not the former, being dominant whites.

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THE pH OF THE BLASTOCŒLE OF ECHINODERM EMBRYOS.

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The development of the calcareous skeleton in Echinoderm embryos has been correlated recently with changes in the hydrogen ion concentration (1, 2, 3). This work was based on the results of Rapkine and Prenant (1) who injected indicator dyes into the blastocœle at the various stages of the developing embryo. They found that the pH of the blastocœlic fluid is 7.3 in the blastula, 8.5 in the early gastrula when the mesenchyme and the calcareous spicules appear, and 7.3 in the pluteus.

More recently (4, 5) it has been definitely shown that an evolution of acid occurs when protoplasm is punctured or torn. In view of this, the experiments for determining colorimetrically the pH of the blastocœlic fluid were repeated on Echinoderm larvæ obtainable at Woods Hole.[1]

The indicator dyes used were those of Clark and Lubs (6) and ortho cresol phthalein. They were prepared with NaOH in .4 per cent. aqueous solutions. All the solutions except that of brom thymol blue when injected proved to be quite innocuous to the embryos.

These dyes in sea water, obtained from the laboratory tank, give a color which indicates a pH of approximately 8.4 uncorrected for salt error and for the possible loss of CO_2 in the hanging drops in which the determinations were made.

I. INJECTION EXPERIMENTS.

Asterias.—The early blastomeres of the *Asterias* egg are so weakly adherent that they would fall apart except for the

[1] We wish to take this opportunity of expressing our appreciation to Dr. Isabella Gordon of the Imperial College of Science, London, for supplying us with late stages of Echinarachnius and Arbacia larvae from cultures which she was growing at Woods Hole during this past summer.

enclosing fertilization membrane. It is not until the eggs reach the 32, 64 or even 128 celled stage that the blastomeres become sufficiently adherent to form a comparatively intact wall about the segmentation cavity. Dyes, injected before the cavity becomes completely enclosed, immediately escape through the gaps between the blastomeres. In later stages the injected dye is retained for a longer period. The color of the dye, however, always begins to fade within a minute or two after the injection and in no case could an appreciable color be detected after 5 to 10 minutes.

The stages injected included the coarse and fine celled blastulæ, early and late gastrulæ, and Auriculariæ of five and ten days growth. In every case the colors of the indicators within the blastocœle were identical with those of the indicators in the environing sea water.

Echinarachnius.—The early blastomeres of the *Echinarachnius* ovum are more adherent than those of the *Asterias*. In the two and four celled stage the hyaline plasma layer is strong enough to press the two blastomeres closely together (7). By inserting micropipette through this layer and between the contiguous blastomeres the injection of the dye solution creates a temporary blastocœle. The blastocœle of early and late blastulæ, gastrulæ and plutei were injected with the dyes. In every case the colors assumed by the dyes were those typical for the pH of the surrounding sea water.

Dyes were also injected into the blastocœle of a pluteus fifteen days old containing a young echinus-rudiment. The dyes diffused through the blastocœle and into its prolongations in the lips and arms of the pluteus. The color of the dye in the blastocœle was always that of the dye in sea water and always faded within a few minutes.

In order to test the effect of changing the pH of the external environment blastulæ, gastrulæ, and early and late plutei, placed in a hanging drop of normal sea water, were injected with dye solutions and were then transferred into acidified sea water the pH of which was still consistent with viable conditions (pH 6.6 ±). The color in the blastocœles changed within a few seconds after immersion to correspond with the new pH. On

being returned to normal sea water, the color quickly changed to that characteristic for the external medium. This procedure could be repeated a number of times until there was not enough appreciable color left within the blastocœle of the living embryos.

Blastulæ were also immersed in sea water whose pH had been brought to 5.4 ± and to 6.0 ±. Three minutes later they were injected with chlor phenol red and brom cresol purple. In every case the color within the blastocœle was the same as that of the dye in the surrounding medium.

Arbacia.—In the *Arbacia* egg the hyaline plasma layer is more prominently developed and much stiffer than in either the *Asterias* or *Echinarachnius* egg. Consequently, the blastomeres are always closely pressed together. The blastocœle appears early as an enclosed cavity; it does not enlarge to the same extent as in the other forms and its cellular wall, during the blastula stage, is much thicker. Therefore, when a pipette is introduced into the blastula a considerable amount of injury usually occurs at the spot where the wall is punctured. The disintegrating material of the injured cells is carried into the blastocœle and the consequent addition of acidified material, which has a pH of 5.4 to 5.6 (5), is sufficient to lower the pH of the internal fluid to that observed by Rapkine and Prenant, viz., 7.0 to 7.3. The color of the dye disappears within several minutes, the time varying with the dye used and the amount injected. This acid reaction in the *Arbacia* at this stage is so readily produced that it was not suspected as an injury phenomenon until experiments were performed on the larger and less readily injured blastulæ of the *Asterias* and *Echinarachnius*. Extra precautions were, therefore, taken to avoid injury and micropipettes were used the tips of which taper rapidly into very slender and elongated shafts. With such a pipette it was found possible to perform the injection with no sign of injury by causing the tip of the shaft to pass between and not through the cells of the blastula at the spot of puncture. The observed pH was then always that of the surrounding sea water.

When gastrulation begins the aboral end of the embryo dilates and the wall of the blastocœle becomes so thin that a puncture produces no appreciable injury. Consequently no special pre-

cautions were necessary and the injections all indicated the same pH as that of the external medium.

Later, when the pluteus stage is reached the archenteron occupies a considerable portion of the blastocœle. When, therefore, the pipette is inserted one is liable to injure the surface of the archenteron. The resulting injury to the archenteron immediately produces sufficient injury to affect the pH of the fluid in the blastocœle. By avoiding injury it was found that the pH of the blastocœle in the various stages of development of the *Arbacia* until metamorphosis is identical with that of the surrounding sea water.

The extreme susceptibility to injury of the cells in the wall of the archenteron is a striking phenomenon. If a pluteus is injected with phenol red and the surface of its archenteron slightly scratched a flash of yellow spreads over the archenteric wall, quickly followed by a return to the red color of the dye in the blastocœle. The cells, themselves, remain colorless and apparently normal. The irritation at the spot of injury seemed to spread from cell to cell and to cause them to produce an acid. This resembles the production of an evanescent acid zone around a mechanically injured starfish egg (5).

The archenteric cavity of a late pluteus was also injected by inserting the micropipette into the blastopore, and, in the few tests performed, the pH recorded was the same as that of the surrounding sea water. If too much pressure is applied the blind end of the archenteron breaks and the injected fluid flows into the blastocœle.

The blastocœle of the so-called bottom swimmers were also injected. These are sluggishly moving blastulæ containing disintegrated material from dying cells which are squeezed out of the blastular wall. The smaller size of these abnormal blastulæ and the accumulated debris in their interior probably account for their inability to acquire the pelagic habits of the normal forms. The injection of dyes into the blastocœle of bottom swimmers indicate a pH below 7.0. As long as disintegration is a continuous process the acid reaction of the blastocœle fluid is maintained. When, however, disintegration ceases and the blastula begins to recover, the pH of the blastocœle gradually rises until it reaches that of the surrounding sea water.

II. Immersion Experiments.

Embryos in all stages of the starfish, sanddollar, and sea urchin were immersed in bowls of normal sea water deeply colored with brom cresol purple, phenol red, meta cresol purple and cresol red. After an immersion of ten minutes to half an hour the embryos were removed, rapidly washed, and placed in fresh sea water. In every case, the dye penetrated the blastocœle and indicated a pH of the seawater. These colored embryos were then placed in sea water having a pH of 6.6 ± (sea water acidified with KH_2PO_4) and the color changed, within a few seconds, to that characteristic of the environing sea water.

III. Conclusion.

The microinjection of dye indicators (see Table), and the

Indicators.	Blastocœle.	Sea water.	pH.
Phenol red	Red	Red	> 7.6
Cresol red	Red	Red	> 8.0
Meta cresol purple	Red wine colored	Red wine colored	> 8.2
Thymol blue	Yellowish green	Yellowish green	< 8.6
Cresol phthalein	Colorless	Colorless	< 8.6

immersion of embryos in sea water colored with dye indicators show that the fluid in the blastocœle of the normally developing embryos of *Asterias forbesii*, *Echinarachnius parma*, and *Arbacia punctulata* has the same pH as that of the environing sea water. This is true for all the stages of the embryos from the time that the blastocœle first appears until metamorphosis.

The fact that the color of the dye in the blastocœle always changes within a few seconds to that typical for the pH of the environing medium whether acid or alkaline, and the fact that the color disappears from the blastocœle of embryos in sea water in which no dye was present, indicate that the wall of the blastocœle of a normal embryo is freely permeable.

The variations in the blastocœlic pH recorded by previous investigators can be accounted for by the ease with which acid is evolved in the sea urchin embryo when the cells of the walls of the blastula and the archenteron of the pluteus are injured.

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THE PERMEABILITY OF FROG SKIN TO UREA.

I. The Influence of NaCl and $CaCl_2$.

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Although there is already an extensive literature relating to cell permeability, many more facts are needed before an adequate explanation of the mechanism involved can be expected. So much of the work reported has been of a qualitative or even of an indirect nature that additional data are needed on the quantitative side, and especially with substances occurring under normal physiological conditions. Accordingly a quantitative study of the penetration of urea through living frog skin under the influence of various reagents has been undertaken. In this paper are reported the effects on the process of different proportions of NaCl and $CaCl_2$. Experiments with other reagents will be reported in subsequent papers.

For a number of reasons urea is an excellent substance for use in studying permeability. It is an organic compound occurring normally in animal tissues and fluids and as such belongs to a class needing much more study. It penetrates tissues with moderate ease, entering neither too quickly nor too slowly for experimental purposes. A sufficient amount of urea for quantitative determination passes through the tissues within a period of time that insures the skin's remaining alive and in healthy condition. This point is of much importance because there is reason to believe that some of the papers on the permeability of frog skin have reported experiments extending over such a length of time that the skins were killed during their progress. On the other hand, the penetration of urea is not so rapid that the amount may not be decreased by agents having an inhibiting effect on permeability. Of practical importance is the fact that direct quantitative determinations may be made so readily on urea.

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There has been little work reported on the effect of NaCl and $CaCl_2$ on cell permeability to non-electrolytes. The number of papers relating to the effect of these salts on permeability to other electrolytes, or concerned with their effects in general on cell permeability, have been so many that only a few typical ones to illustrate the different kinds of studies may be mentioned here. Osterhout (1) showed that there is a difference of conductivity with different proportions of NaCl and $CaCl_2$ in the case of various plants, and he proved that this also holds true for frog skin (2). However, in a more recent paper (3) he has expressed the view that an increase of conductivity does not necessarily indicate a greater permeability to ions in the absence of electric currents. Harvey (4) noted that NaOH enters Spirogyra cells more easily from a NaCl solution than from a mixture of NaCl and $CaCl_2$. R. Hamburger (5) found that artificial edema may be produced by solutions lacking calcium. In 1909 R. S. Lillie (6) reported that pure isotonic solutions of sodium salts increase the permeability of the pigment-containing body cells of Arenicola larvæ, while this effect is prevented by addition of $CaCl_2$. Many other papers by the same author refer to similar effects of these salts in relation to permeability. In a paper dealing with artificial parthenogenesis, Lillie and Baskervill (7) assumed that the greater effect of NaCl as compared with mixtures of NaCl and $CaCl_2$ in causing development is due to the increase of permeability in the former case. Since it has been so often assumed that NaCl will increase the permeability of tissues for all substances and that certain proportions of $CaCl_2$ will decrease permeability, it seemed desirable to ascertain how much the salts in question might influence the permeability of frog skin for a non-electrolyte, urea.

The skin used was obtained either from the bull frog, *Rana catesbiana*, or from *Rana virescens*, and was removed with as little injury as possible. Usually it was tied over the end of a glass tube 16 mm. in diameter. In a few experiments the skin from a whole leg tied at the distal end to form a sac was used. Measured amounts of the salt solutions under investigation were put into the tube or sac. The skin was then immersed in a bottle containing 50 to 100 cc. of urea solution, an amount

large enough to prevent any significant change of concentration during the experiment.

The skins were used in symmetrical pairs; so that with each pair of skins taken from corresponding parts of the two sides of the frog, one was treated with NaCl (0.7 per cent.), and the other with isotonic $CaCl_2$ (1.1 per cent.), used either alone or in combination with the NaCl solutions. All references to salts in this paper are to solutions of the above mentioned concentrations.

After a convenient interval of time, varying usually from two to seven hours, the amount of urea that had penetrated the skin was measured by the Van Slyke-Cullen method (8). Numerous experiments were performed because conclusive results could be obtained only by frequent repetition of each experiment in order to compensate for the high degree of variability in the skin, in part seasonal, in part individual. With frogs that had been in captivity for some months, and especially with frogs that had developed "red-leg," no significant difference in the permeability could be obtained. However, even in these skins such slight differences as were noted were virtually always in the same direction as in normal skin.

In most of the experiments a 5 per cent. solution of urea was used, as lower concentrations did not yield significant differences. In order to subject, as far as possible, all of the cells of the skin to the desired proportions of salts, the urea was usually dissolved in the same isotonic salt mixtures as were used on the other side of the skin. Although the objection might be raised that this strongly hypertonic solution penetrates readily by reason of injurying the tissues, there is evidence to the contrary. Skins that have been killed allow equal quantities of urea to pass whether NaCl or NaCl and $CaCl_2$ is used; the fresh skins, however, show marked differences of behavior under the conditions of the experiments. Furthermore, if an isotonic solution of urea, *i.e.*, 1.5 per cent., is used with one of each pair of skins in a series, and the other skin is treated with 1.5 per cent. urea solution brought up by means of glucose to the same osmotic pressure as a 5 per cent. urea solution, there is no essential difference in the passage of urea in the two series. For these reasons it is felt that the experiments reported are no more

open to objection than any experimental case where solutions other than lymph and water are used on the inner and outer sides, respectively, of the frog skin.

Following are the results from several typical experiments. When the inner side of the skin is against the tube and so in contact with the pure salt solution, while the outer surface of the skin is in contact with urea, the direction is designated as "normal" and the reverse direction as "turned."

The data in Table I. show the kind of results obtained when skins from whole legs were used. The skins were turned in the "normal" direction and each contained 5 cc. of the solution indicated. They were washed in the respective solutions for $1\frac{1}{4}$ hours before the experiment started. Three per cent. urea dissolved in the same salt solution as used on the inside of the skin was employed in each case. Penetration was allowed to continue three hours.

TABLE I.

Date of experiment 10/26/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	34.02	35.88
10	90	16.26	30.60
15	85	15.66	33.36
25	75	23.82	28.02
50	50	25.14	30.30
100	0	20.76	23.88

Table II. presents data typical of many experiments carried out with skins in the "normal" direction over 16 mm. tubes. They were washed for fifty minutes in the respective salt solutions and then immersed in 5 per cent. urea dissolved in the same salt solutions as used on the inside of the tube. Penetration continued four hours.

Table III. gives data obtained with the skins in the reverse relation, *i.e.*, "turned," over 16 mm. tubes. The skins were washed for one hour in the respective solutions before the experiment started and were then immersed in 5 per cent. urea dissolved in the same isotonic salt solution as was used inside the tube in each case. The penetration continued five hours.

TABLE II.

Date of experiment 11/3/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	13.32	13.68
10	90	11.88	16.62
15	85	14.04	18.90
25	75	12.60	16.08
50	50	11.16	15.24
100	0	12.66	11.82

TABLE III.

Date of experiment 11/11/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	5.88	14.04
10	90	1.44	5.28
15	85	1.26	6.48
25	75	3.90	9.18
50	50	4.56	7.44
100	0	6.72	4.44

Table IV. is another typical series with the skins "turned" over 16 mm. tubes. The skins were washed for one hour in the respective salt solutions and then immersed in 5 per cent. urea dissolved in water. Penetration continued for three hours and twenty minutes.

TABLE IV.

Date of experiment 12/12/22.

Isotonic $CaCl_2$ and NaCl.			Isotonic NaCl.
$CaCl_2$.	NaCl.	Mg. Urea Penetrating.	Mg. Urea Penetrating.
5%	95%	6.72	8.28
10	90	9.84	9.18
15	85	7.68	10.62
25	75	3.66	7.08
50	50	4.26	7.44
100	0	2.04	5.76

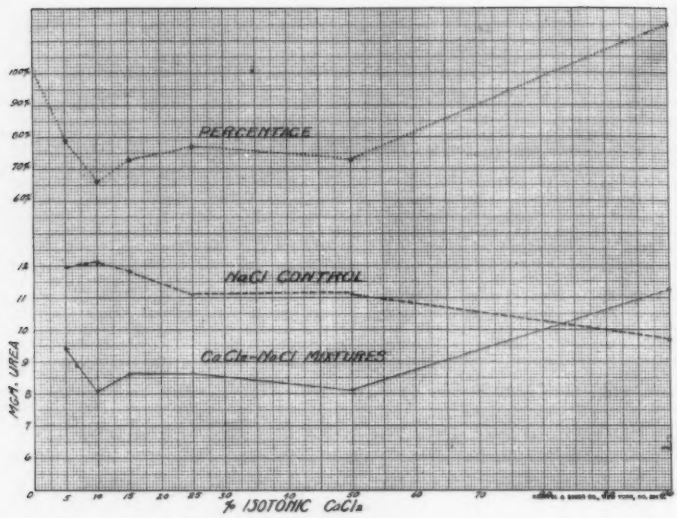

CHART I. The chart represents the composite results obtained in ten experiments.

The solid line represents the amount of urea in milligrams penetrating the skins in the $CaCl_2$-NaCl mixtures.

The broken line represents the amount of urea in milligrams penetrating the skins in the control experiments when NaCl solution alone was used.

The dotted line represents the percentage of permeability on the basis of the permeability with pure 0.7 per cent. NaCl solution alone, which is taken to be 100 per cent.

The minimum penetration of urea is obtained with very different percentages of $CaCl_2$ in the various series of experiments. This might be expected from the variability of the tissue to which reference has already been made. With a material like frog skin, composed of many layers of cells, there can be obtained only a rough approximation of the actual proportions of salts in contact with the majority of cells. When one is using skin comparatively permeable to the salts in question, and has washed it in the salt solution for some time before subjecting it to the penetration of urea, the proportions of salts in contact with the cells in the interior of the tissue approach more closely to those of the original solution than is the case at other times. With tissues relatively impermeable to the salts, it takes a

higher concentration of $CaCl_2$ in the external solution to produce a concentration of about 10 per cent. in the region of the greater number of the cells. In support of this idea the figures presented show that sometimes the minimum amount of urea penetration was obtained with 25 per cent. or 50 per cent. $CaCl_2$, or even in one case in pure isotonic $CaCl_2$ solution, as in Table IV., quoted. When the urea was dissolved in the same mixture of isotonic salt solution as that used on the other side of the skin, the concentration of $CaCl_2$ within the inner strata of the skin was probably much more easily reached, and the most striking differences in the permeability to urea was shown with experiments thus carried out.

Summary and Conclusions.

Experiments were carried out on the effects of NaCl and $CaCl_2$ alone and in combination upon the permeability of frog skin to urea, with the following results.

Frog skins bathed in isotonic solutions containing varying proportions of $CaCl_2$ and NaCl showed less permeability to urea than did the controls of matched skins bathed in pure isotonic NaCl.

The minimum amount of penetration of urea, while varying much with different skins, was obtained on an average with about 10 per cent. of a 1.1 per cent. $CaCl_2$ solution plus 90 per cent. of a 0.7 per cent. NaCl solution.

In many cases, isotonic $CaCl_2$ solutions alone as compared with pure NaCl solutions increased the permeability to urea.

The writer is indebted to Dr. M. H. Jacobs for suggesting the study of urea with frog skin and for his continued interest and criticism.

This work was first undertaken at the University of Pennsylvania during the year of 1922–23, but could not be completed until facilities were obtainable again at the University of Texas during 1925–26. This accounts for the long break in the date of the experiments.

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THE PERMEABILITY OF FROG SKIN TO UREA.

II. The Effect of Dextrose and Sucrose.

M. L. BASKERVILL.[1]

Introduction.

Loeb (1) in a series of papers on the passage of potassium through the membranes of *Fundulus* eggs demonstrated that this substance would pass through in the presence of other electrolytes, but failed to do so with various non-electrolytes. The explanation that he offered and termed the "salt effect" could apply only to electrolytes.

Since the experiments of Loeb on potassium, there has been a good deal of other work comparing the permeability of tissues to electrolytes in the presence of other electrolytes and in the presence of non-electrolytes. Hoeber and Memmesheimer (2) showed that basic dyes enter red blood corpuscles if dissolved in salt solutions, but not if they are dissolved in sugar or glycocoll solution. Wertheimer (3) confirmed the need of electrolytes for the penetration of basic dyes through frog skin. Hiruma (4) reported that blood corpuscles take up less ammonium and alkaloid salts from a sugar solution than from a salt solution, while more salicylate or thiocyanate is taken up from the sugar solution. With frog muscle he found that basic dyes, alkaloid salts, salicylate, and thiocyanate have their penetration favored by sucrose as have also iodide and acid. The sugar checked only sodium hydroxide.

Embden and Adler (5) noted that if frog muscles were immersed in isotonic sugar solution, phosphoric acid would pass out, and the muscles would lose their contractility, although even after a stay of several hours in the sugar solution they would recover upon being again placed in Ringer's solution. Phosphoric acid was also given off in Ringer's solution if the muscles were fatigued or injured. The authors believed there

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was a direct connection between the permeability for phosphoric acid and a non-functional, *i.e.*, an injured, condition of the muscle.

Among the papers describing the conditions for the permeability of non-electrolytes, probably no other is so free from the objection that the permeability reported might be due to injury as an article by Reid (6) in which he found that absorption of glucose from the intestine is favored by the presence of salts. Hiruma (4) in the paper referred to above reported that the permeability of blood corpuscles for urea is not decreased but probably increased by the presence of other non-electrolytes.

Wertheimer (3) claimed that if frog skin had its inner surface in contact with solutions of non-electrolytes more glucose would penetrate than was the case when Ringer's solution was employed in the same way. In an earlier paper (7) he had reported that sugar passed through the frog skin into Ringer's solution if the sugar was in contact with the inner surface of the skin and the Ringer's solution on the outer surface, while no sugar passed if the direction of the skin was reversed. The durations of the experiments were fifteen hours or more. Bauer (8) objected that Wertheimer secured more permeability to sugar under the conditions that are more injurious to the skin; that is, with sugar in contact with the inner surface of the skin. In his last paper Wertheimer (9) has again stated that if a non-electrolyte bathes the inner surface, frog skin is permeable to sugar; if an electrolyte bathes the inner surface, it is impermeable. This is just the condition that Bauer maintains is most injurious, so some evidence as to the state of the skins is needed.

Most of the writers mentioned confirm the influence of electrolytes in favoring the permeability of tissues to other electrolytes. The evidence in regard to their effect on the permeability of tissues to non-electrolytes is both more meagre and more conflicting. Since the permeability of frog skin to urea has been shown in the preceding paper (10) to be decidedly influenced by the kind of salts in contact with the tissues, the writer wished to see what effect the absence of electrolytes, or the presence of another non-electrolyte would have on the penetration of the non-electrolyte, urea.

The permeability of frog skin to urea as influenced by electrolytes and by non-electrolytes may readily be ascertained, since direct chemical determinations of the amount of the substance passing through the skin may be made. Urea has the further advantage of penetrating so readily that the tissues may maintain their vitality through the period of experimentation. Hence it seemed of interest to find how much urea will pass through frog skin when it is bathed in Ringer's solution and when it is bathed in sugar solutions.

In experiments carried out during the winter of 1922–23 much less urea penetrated into sucrose solutions than into Ringer's solution. As soon as facilities for the work could be secured again, the experiments were repeated and extended to dextrose. While the results quoted are in most cases the more recent ones, they do not differ in kind from those obtained several years previously.

Materials and Methods.

The procedure was similar to that described in the foregoing paper (10). Matched pairs of frog skin were put over 16 mm. tubes. Into one tube of each pair was measured a convenient amount, usually 2.5 cc. of the sugar solution; into the other, the same amount of Ringer's solution. Each tube was then placed with its other surface bathed by the urea solution. All solutions referred to were isotonic except the urea solution. which was five per cent. In preparation for the experiment, each skin was usually washed about an hour in the particular solution that was to be used on the inside of the tube. The exposure to urea was continued for varying times. Series of experiments were carried out with both dextrose and sucrose.

When the study of the permeability of frog skin to urea was begun, much time was spent in trying to discover if the orientation of the skin made any difference in its permeability to urea. Przylecki (11) has reported that urea penetrates frog skin equally well in both directions. Under the conditions employed in this paper it was found to penetrate readily in either direction, while no marked difference in the rate of penetration in the two directions was shown except in tubes where a solution used might be more injurious to one side of the skin than to the

other. For example, if sugar solution was in contact with the inner surface of the skin, after five or six hours urea penetrated much more rapidly from the outer surface than was the case if the reverse relations held. For this reason, while results are reported below for both directions, it is considered that the more significant ones were obtained with skins that had their inner surfaces in contact with urea solution, which seems fairly harmless for the tissue, and the outer surface in contact with the sugar solution. The use of solutions abnormal for the skins is discussed in detail in the recent article by Bauer (8).

Data.

Throughout the tables "normal" direction indicates that the inner surface of the skin was toward the tube and hence in contact with the sugar or Ringer's solution respectively, and the outer surface in contact with the urea; while "turned" indicates the reverse relation. The amount of urea penetrating was determined by the Van Slyke-Cullen method, and is expressed in milligrams in all tables. The figures for matched skins are given in parallel columns.

The data outlined in Table I. show the kind of results always obtained when pure sugar solution and Ringer's solution were compared with the skins turned in the two directions. The skins were washed forty minutes in Ringer's or dextrose solution, respectively. Penetration was allowed to continue for three hours and forty minutes.

Table I.

4/2/23.

Isotonic Dextrose Solution.	Ringer's Solution.
Skins "Normal" Direction.	
Mg. Urea.	Mg. Urea.
5.88	9.72
4.50	9.00
5.40	10.20
Average 5.26	Average 9.84
Skins "Turned."	
2.16	14.46
2.46	15.60
2.40	17.52
Average 2.34	Average 15.86

In Tables II. and III. are presented typical data showing the penetration of urea dissolved in varying concentrations of dextrose. The Ringer's and dextrose solutions were in contact with the inner surfaces. The percentage of isotonic sugar solution is indicated in the first column; it is made up to 100 cc. with Ringer's solution in each case. The skins were washed for one hour in the respective solutions. Penetration was allowed to continue for three hours. The skins were "turned."

TABLE II.

11/30/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	7.32	7.86
75	5.16	7.44
85	5.22	8.34
90	7.50	7.86
95	1.68	9.48
100	0.96	8.04

TABLE III.

12/1/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	3.72	5.88
75	2.76	7.74
85	4.08	6.66
90	4.80	7.02
95	2.64	8.70
100	1.20	4.74

Results with the skins in the reverse relation are given in Tables IV. and V. The skins had the inner surface in contact with the Ringer's or sugar solutions, and the outer side in contact with five per cent. urea. The skins were washed for one hour in the respective solutions. Penetration continued for three hours, and the skins were in the "normal" direction.

The results with sucrose differed no more from those obtained with dextrose than did results with either sugar alone when different skins were employed, as is indicated by the data

recorded in Tables VI. and VII. The skins were "turned" and washed for one hour in the respective solutions. The time of penetration was three hours in all cases.

TABLE IV.

12/3/25.

Per Cent. Dextrose.	Isotonic Dextrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	5.10	5.28
75	4.32	7.80
85	2.76	4.86
90	2.52	3.96
95	4.20	5.64
100	2.34	5.76

TABLE V.

12/4/25.

Per Cent. Dextrose.	Isotonic Dextrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	7.08	6.12
75	4.50	8.10
85	6.12	6.48
90	6.88	6.90
95	5.82	7.80
100	4.44	7.74

TABLE VI.

3/2/26.

Per Cent. Sucrose.	Isotonic Sucrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	7.26	9.72
75	8.46	9.06
85	6.00	8.58
90	2.34	9.06
95	3.96	8.34
100	0.30	8.22

TABLE VII.

3/1/26.

Per Cent. Sucrose.	Isotonic Sucrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	6.24	6.84
75	9.12	10.50
85	7.02	8.58
90	5.70	9.18
95	4.98	10.68
100	0.54	8.76

Reference has been made to the greater permeability to urea in sugar solutions if the exposure is prolonged beyond three or four hours. Table VIII. contains typical results showing more penetration of urea in the solution of pure dextrose than in the

TABLE VIII.

2/27/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	4.14	6.60
75	3.30	9.72
85	4.80	7.98
90	3.18	3.18
95	4.20	4.98
100	10.32	6.66

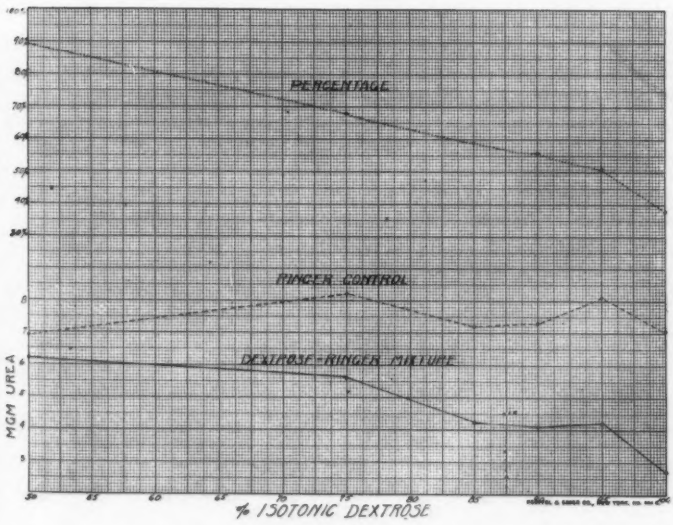

CHART I. The chart represents the composite results obtained in ten experiments.

The solid line represents the amount of urea in milligrams penetrating the skins in the Dextrose-Ringer's mixtures.

The broken line represents the amount of urea in milligrams penetrating the skins in the control experiments when Ringer's solution alone was used.

The dotted line represents the percentage of permeability on the basis of the permeability with Ringer's solution alone, which is taken to be 100 per cent.

control of Ringer's solution; and less penetration than into the controls in solutions containing dextrose with sufficient amounts of Ringer's to protect the skin against too rapid death. The skins were in the "normal" direction; washed for one hour and forty-five minutes; penetration five hours.

In order to compare the penetration of urea through dead frog skin with that through living skin, the skins were killed in several ways: by exposing to 60 per cent. ethyl alcohol; to 5 per cent. formalin; or to 0.5 per cent. sodium fluoride, from forty-five minutes to one hour. Often skin that had been killed in alcohol or sodium fluoride showed the passage of more urea into the pure sugar solution than into Ringer's solution. The reason for this behavior is not known, unless it is that the constituents of the skin are not "fixed" so thoroughly by these chemicals as by formalin, and therefore the subsequent treatment with sugar causes more disorganization in the skin and more penetration than could occur in the latter case. This point is of chief interest in showing that the more viscous sugar solutions do not slow the rate of diffusion of urea more than does the Ringer's solution.

In Tables IX. to XII. are presented data typical of results obtained in a large number of experiments with dead skins. The skins were washed in the sugar or Ringer's solution respectively before and again after being killed in the solutions indicated. Penetration of the urea continued from three to three and a half hours.

TABLE IX.

3/19/26.

	Isotonic Sucrose + Ringer's Solution.		Ringer's Solution.
	Per Cent. Sucrose.	Mg. Urea.	Mg. Urea.
Killed in alcohol.....			
" Normal" direction.....	50	15.66	10.68
	75	18.90	10.89
	100	21.12	10.50
"Turned".....	50	15.99	9.51
	75	19.17	10.59
	100	19.71	11.16

TABLE X.

4/27/26.

	Isotonic Sucrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Normal" direction	1.74	2.07
	1.11	1.98
Killed in alcohol:		
"Normal" direction	19.83	12.63
"Turned"	17.49	11.58
Killed in NaF:		
"Normal" direction	5.82	5.04
"Turned"	6.45	4.95

TABLE XI.

5/12/26.

	Isotonic Dextrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Turned"	4.56	7.32
Killed in NaF:		
"Normal" direction	4.32	1.98
"Turned"	3.84	3.78
Killed in formalin:		
"Normal" direction	7.56	7.74
"Turned"	8.16	6.84
Killed in alcohol:		
"Normal" direction	24.48	18.48
"Turned"	26.82	21.78

TABLE XII.

5/18/26.

	Isotonic Dextrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Normal" direction	3.54	5.16
Killed in NaF:		
"Normal" direction	12.74	10.08
"Turned"	5.58	3.84
Killed in formalin:		
"Normal" direction	3.48	3.00
"Turned"	3.90	5.46
Killed in alcohol:		
"Normal" direction	14.76	14.76
"Turned"	18.72	15.30

Discussion.

The experiments show that in the case of the shorter exposures the passage of urea through frog skin was checked by the presence of sugar. After about six hours, the time differing with different skins, the skins in a pure sugar solution were greatly injured, especially if the sugar was in contact with their inner surfaces. As a rule, skins in mixtures of sugar and salt solutions survived in proportion to the amount of salt present. In contrast to these conditions, skins bathed on the inner surface by Ringer's solution survived 24 hours and upward. Accordingly, when skins were exposed to the solutions for six hours or more, the skins in contact with the solutions containing the larger proportions of sugar were the more injured and permitted more urea to pass. Skins that had been killed in various ways showed more nearly the same degree of permeability in Ringer's and in sugar solutions.

As in all work on permeability of cells and tissues, it is necessary to distinguish carefully between the permeability caused by injury and that normal to tissues. It is not claimed that in these experiments the skins remained entirely uninjured during the time—three or four hours—that constituted the minimum exposure to the solutions used, which are quite abnormal for frog skin; but it is believed that the skins remained in a sufficiently healthy condition for this length of time to exercise a considerable degree of the selective permeability that is characteristic of living tissues. In spite of the fact that skins exposed to the sugar solutions even for the shorter periods would be expected to be proportionately more injured than those exposed to Ringer's solutions, more permeability to urea was shown in the latter condition.

Because of these results on urea it may be concluded, therefore, that the favorable effects of salts on the penetration of dissolved substances is not limited to the passage of other electrolytes as some authors have assumed, but applies to certain non-electrolytes as well. This is not surprising considering the universal presence and the known physiological importance of the electrolytes in living cells and tissues.

Summary.

1. Isotonic dextrose or sucrose solutions, as compared with Ringer's solution, check the passage of urea through frog skins if the exposure to the solutions is not so prolonged that the skins are injured.

2. When the skins are kept in contact with the solutions for such a length of time that those in the sugar solutions are injured or killed and those in Ringer's solution are still in a healthy state, then more urea may penetrate in the presence of the sugar solution.

3. Mixtures of isotonic sugar solution and Ringer's solution check the penetration of urea in proportion to the amount of sugar present when that amount reaches a concentration of 50 per cent. or more.

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THE CLOACA AND SPERMATHECA OF *GYRINOPHILUS PORPHYRITICUS*.[1]

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INTRODUCTORY.

The first observations on the cloaca and spermatheca in American urodeles were made by Kingsbury in 1895. He described seven forms. Apparently the development of the spermatheca did not receive attention until recently, when Koehring ('25) described it in *Eurycea bislineata*. In the present study a description of the form and development of the cloaca and spermatheca in *Gyrinophilus porphyriticus* has been attempted, and certain observations have been made which throw some light on the question of time of mating and ovulation in this form.

The material consists of fourteen fully developed adults, six animals which had undergone metamorphosis but were not fully developed, and six larvæ. After fixation in Bouin's fluid, formalin or alcohol, the cloacæ of all the animals except one adult were imbedded in paraffin and sectioned serially, usually at 12 μ. The remaining adult was studied under the binocular dissecting microscope. The ovaries of eleven animals were sectioned to determine size of ova and deposition of yolk at different seasons. A single specimen each of *Eurycea*, *Plethodon glutinosus*, *Plethodon cinerius*, and *Diemyctylus* were sectioned for comparative study.

OBSERVATIONS.

The Cloaca in Adults.—The mid-sagittal section (Fig. 11) shows the main features of the cloaca, namely, a well marked diverticulum located in the dorsal wall above the vent, ahead of this a narrowed portion, which, as it is followed in a cephalic

[1] I desire to express my gratitude for advice and guidance, as well as for material, to Dr. R. R. Humphrey, who suggested this study.

direction, expands both dorsally and ventrally. In the dorsal recess thus formed, the uro-genital papillæ are located; at the same level the cloaca communicates ventrally with the bladder.

The cloaca is a slit-like cavity, and is thrown into well-marked folds, of which, however, only those on the dorsal wall are significant. In the middle third of the cloaca there are two folds in this location, with a median dorsal slit between them (Figs. 4 and 5). The slit extends as far forward as the opening of the spermatheca (Fig. 6), cephalic to which the two folds are fused into one (Fig. 7). The folds, including both their distinct and fused portions, correspond to what Kingsbury (1895) has called in other species, the dorsal elevation. The extent of the elevation is indicated on the sagittal section (Fig. 11). The shelf-like area seen below the diverticulum in the same section corresponds to the freely projecting papilla described by Kingsbury in *Plethodon;* in *Gyrinophilus*, however, it is not free like a tongue, but is merely the dorsal wall of the cloaca below the diverticulum, and is continuous across from one lateral wall to the other. Since the mid-sagittal section passes between the two limbs which compose the caudal two thirds of the dorsal elevation, these limbs or folds do not appear in this section.

Cephalic to the fused portion of the dorsal elevation, this structure is seen to break up into three folds (Fig. 8), the lateral of which merge into the lateral cloacal walls, while the median, a slender fold, after continuing forward a short distance (Fig. 9), disappears abruptly as the cloaca expands dorso-ventrally. The entire elevation is pigmented, especially in the region of its two limbs caudal to the opening of the spermatheca.

According to Kingsbury, both dorsal and ventral glands may be present in the cloaca of the urodele female; but they are quite variable, for, of seven forms described by him, *Diemyctylus* has ventral glands only, *Necturus* and *Amblystoma* have both well developed, *Eurycea* has ventral well developed and dorsal rudimentary, and the *Plethodons* and *Desmognathus* have neither. In *Gyrinophilus* females the ventral glands alone are well developed. They lie, one group on each side of the mid-line, in the ventral and lateral walls of the cloaca (Fig. 4). The area occupied by glands extends from the cephalic end of the dorsal

diverticulum caudally, to the opening of the spermathecal tube cephalicly, or approximately between the levels of Figs. 4 and 5 indicated on the sagittal section (Fig. 11). The glands are tubular in form and somewhat tortuous. Their epithelium is of the mucous-secreting type. Their narrow ducts open into the ventral and lateral walls of the cloaca, both on the summits of and between folds.

In addition to these functional glands, a group of rudimentary glands is found, opening into the mid-dorsal slit, caudal to the spermatheca. These are inconstant and variable in number. They are sometimes absent; sometimes only a single tubule is found on one side, or there may be three or four pairs.

The epithelium of the cloaca is a stratified squamous just inside of the vent, throughout the caudal portion of the cloaca, in the dorsal diverticulum, and on the elevation as far forward as the opening of the spermatheca. This gives way to a stratified cuboidal epithelium, which is two layers deep on the ventral wall of the cloaca opposite the spermathecal tube, and three or four layers on the elevation, in the median slit, and in the cephalic expansion of the cloaca. This stratified epithelium, in turn, gives way in the gut to a tall columnar variety with goblet cells, and in the bladder to a simple cuboidal.

A comparison of the cloaca of *Gyrinophilus* with that of other forms, relates it most closely to *Eurycea* and the Plethodons. *Eurycea*, though lacking the diverticulum, has the dorsal elevation divided into two limbs with the median dorsal slit between them; and the Plethodons, in which species the elevation has no dorsal slit but is single throughout, have the dorsal diverticulum. In *Plethodon glutinosus*, this diverticulum extends forward above (dorsal to) the spermathecal region in my specimen. Kingsbury describes it as of considerable extent.

The Spermatheca in Adults.—The spermatheca, or sperm-storing organ, definitely associated in urodeles with internal fertilization, is, in *Gyrinophilus*, a complex structure, located dorsal to the cloaca, and consisting of a branched common tube and numerous smaller tubules. The common tube, constricted slightly near its mouth, and pointing almost directly dorsad from its opening between the limbs of the dorsal elevation,

expands into two short but thick lateral branches. These branches curve both latero-ventrad and caudad (Figs. 5 and 12). Into them and into the distal end of the common tube open small flask-shaped tubules, with long slender necks and bulbous, blind ends; these are bilaterally arranged in two groups, varying from fifteen to twenty-five in each group in different animals. Rarely a tubule is double: *i.e.*, one slender neck terminates in two bulbs. The tubules point caudad, dorso-caudad, laterad and ventrad, but not cephalad and not directly dorsad. Spermatozoa, when abundant, are found in the common tube, its branches, and in both neck and bulb of the tubules (Fig. 12). In the bulbs they are arranged in a whorl (Fig. 13), lying side by side, heads all pointing in the same direction. A similar cluster is sometimes found streaming into the neck of a tubule, but in other necks and in the common tube, there are only a few stray sperms. When sperms are scanty, they are tangled and disordered, as though they had been brushed off when the larger clump was expelled by muscular contraction, and are found in the lateral branches of the common tube, and in some tubules, but are absent from others.

Surrounding the organ as a whole, as well as each tubule and the common tube, are numerous plain muscle fibers. Pigment cells, containing coarse black or brown granules, are found to be most abundant around the expanded end of each tubule, moderately abundant in the dorsal elevation, and scanty beneath the epithelium of the rest of the cloaca, among the muscle fibers of its wall, and about the ureters.

The epithelium of the spermatheca is stratified in the common tube and its branches, the surface layer of columnar cells becoming progressively taller toward the upper end of the tube. It becomes a simple, low columnar in the necks of the tubules, and a simple, tall columnar in their expanded ends. In this location the cytoplasm takes a deeper eosin stain than in any other part of the organ, and is finely granular.

The size of the spermatheca in animals of 16.5 cm. length is approximately 1.05 to 1.20 mm. in each of its dimensions (transverse, cephalo-caudal and dorso-ventral, including the common tube). If larger, it is the transverse diameter which is most

likely to be increased. In the smallest adult in the series, measuring 12.0 cm., the dimensions were 1.0 mm. each. The above measurements were made from serial sections of the organ.

Discussion.

An examination of the spermatheca in different species suggests that the complex forms found in *Eurycea*, *Gyrinophilus*, the Plethodons and *Desmognathus* are developed from the simpler forms (Kingsbury). In *Diemyctylus* and *Necturus* each tubule opens separately into, or along the margin of a dorsal depression or slit in the cloaca; this slit in *Diemyctylus* is partially divided into two by a low ridge. The tubules are numerous (25–40), and flask-shaped. In *Amblystoma* the tubules open, not into a dorsal slit, but into a depression in the dorsal wall, and instead of being scattered, are aggregated and surrounded by plain muscle. *Gyrinophilus*, retaining the numerous tubules, presents, instead of a depression, a tube, constricted at its neck. Its branching reminds one of the definitely bilateral character of the organ in *Diemyctylus*. *Eurycea*, with its occasionally branched [1] common tube, resembles *Gyrinophilus*, but has fewer tubules (12–14). The Plethodons and *Desmognathus* present a further reduction in number of tubules (4–6), and a loss of the characteristic flask shape.

If these forms be arranged in a phylogenetic series on the basis of this one feature, the form of the spermatheca, the tendency in the ascending scale seems to be, first, the aggregation of the tubules from a scattered position along a dorsal slit, to a group around a common tube, the expanded end of which may or may not be branched; and second, the reduction of the number of tubules and loss of their flask shape. Correlated with these tendencies in the spermatheca, the importance of the glands is diminished. If we except *Diemyctylus*, the lower members (*Necturus* and *Amblystoma*) have both dorsal and ventral glands, though Kingsbury states that the epithelium of

[1] Kingsbury does not describe the branching of the common tube in *Eurycea*, but I find it in my specimen, and Koehring, who finds it only in her largest animal (90 mm.), regards it as a late development. Possibly the occasional occurrence of branching in this form represents a transition between the branched common tube of *Gyrinophilus* and the unbranched of the Plethodons.

the dorsal glands of *Necturus* resembles that of the cloaca, rather than that of other cloacal glands. *Eurycea* and *Gyrinophilus*, which stand mid-way in the series, have functional ventral glands, and rudimentary dorsal glands. The Plethodons and *Desmognathus*, at the top of the series, have neither dorsal nor ventral glands. Kingsbury suggests that spermathecal tubules are modified dorsal glands. Apparently, as the spermatheca departs farther from the simple tubules from which it was derived, the gland tubules are suppressed; the dorsal group first, and later the ventral.

The possible significance of pigment cells as attracting or being attracted by spermatazoa is discussed by Kingsbury. There appears to be some correlation between the pigmentation of the spermatheca in the female and the testes and ducts in the male,[1] for *Eurycea*, *Gyrinophilus*, the Plethodons and *Desmognathus*, all show abundant pigmentation in both sexes; *Amblystoma* none in either sex; *Diemyctylus* moderate in the female, and little in the male. In *Necturus* the spermatheca is free from pigment, and testes and ducts only very slightly pigmented.

Pigment is absent from the cloaca of *Gyrinophilus* in the earliest stages of development of the spermatheca, but appears in increasing amount with advancing development. In this series it is found in fair abundance in a small female with well developed spermatheca. The size of this animal (12 cm.), the absence of debris in the organ, and the minuteness of the ova speak against the animal ever having mated. We may conclude, therefore, that the deposition of pigment is not in response to the presence of sperms, but is a normal developmental phenomenon, and may serve to attract sperms, as suggested by Kingsbury.

Three questions naturally suggest themselves in a study of the spermatheca: first, the probable time of mating, second, the time of ovulation, and third, the length of time sperms may remain in the organ. The following generalizations, drawn from facts tabulated in Table I., have some bearing on these problems in *Gyrinophilus:*

[1] Information regarding pigmentation in the males of these species is supplied by Dr. R. R. Humphrey.

TABLE I.*

SHOWING PRESENCE OF SPERMS AND SIZE OF OVA IN *Gyrinophilus* FEMALES AT VARIOUS MONTHS.

(The males have no sperms in the vasa deferentia from approximately June 1 to September 15.)

In the following table (1) signifies that the cloaca, or ovary, was not available for examination; (2) that, although the ovary was not available, the ova were recorded as "minute" at the time of capture of the animal; (3) that two animals of the same size were captured on the same date, and gave identical findings; + that the tail of the animal was broken, and the actual length was greater than that given.

Date.	Presence of Sperms in Spermatheca.	Size of Largest Ova in Mm., and Deposition of Yolk.	Length of Animal in Cm.
Mar.....	(1)	0.41; yolk absent	16.7
Apr. 6..	Absent (spring captivity)	2.50; " abundant	16.5
June 19..	Absent	0.68; " scanty	14.3 +
June 26..	Abundant	2.75; " abundant	17.0 +
July 1..	Moderate	0.97; " moderate	16.5(3)
July 1..	Scanty	(1)	15.5
July 24..	(1)	0.32; yolk absent	(1)
July 25..	Absent	(1)	14.5
Aug. 15..	Abundant	2.70; yolk abundant	13.5
Aug. 15..	Absent	1.00; " scanty	13.3
Sept. 25..	Scanty	1.7 ; " abundant	16.5
Sept. 26..	Absent	(2)	12.0
Nov. 19..	Abundant	2.25; yolk abundant	17.5
Nov. 19..	Absent	(2)	15.5

1. The smallest animal in which sperms or large ova were found, measured 13.5 cm.; therefore, only animals of this size and larger were considered in studying the question of mating and ovulation. In smaller animals (12.0 and 13.3 cm.), the spermatheca, though well developed, was found perfectly clean and free from debris.

2. The presence of sperms in the spermatheca does not depend upon season, for they were abundant in June, August and November specimens (one in each month), moderate or scanty in July, scanty or absent in September, and absent in some animals in every month in which any specimens were captured.

*Two additional animals have been examined, both killed in May. The first, measuring 15.3 cm., was captured and killed May 2d. Ova measured 1.0 mm., yolk was scanty, sperms were absent. The second was longer than 15.0 cm., and was killed May 3rd after two weeks of captivity with a male. Ova were minute, yolk absent, and sperms absent.

3. Ova, presumably large enough for ovulation (2.25–2.75 mm.), appeared in April, June, August and November. Since the August animal was small and possibly ovulating for the first time, the other three might point to spring and fall ovulations in this species; but there is no sequence in size of ova leading up to April, June, or November. Possibly each animal ovulates but once a year, and food, temperature and individual habit may determine the time.

4. Of the four animals showing large ova, sperms were abundant in the three taken in June, August and November. The fourth animal, killed in April, had been kept in captivity for several weeks, and would thus have been prevented from mating during that period.

5. Sperms were not found in abundance except in animals nearly ready to ovulate. Whether or not normal sperms might be retained long enough to fertilize ova at the following ovulation, would be impossible to say from this series. An animal taken in September with ova two thirds of the maximum size (ready to ovulate in October or November?) had a few sperms remaining.

6. Mature sperms are absent from the vasa deferentia of the male from approximately June 1 to September 15.[1] Males are, therefore, incapable of functional mating during this period. The sperms found in females between June and September must have been acquired before June—probably in early spring or the preceding autumn. Of especial interest is the abundance of apparently normal sperms in the female taken in August. We may conclude that sperms can be stored in the spermatheca for at least two and a half months, and most probably for a far longer period.

The interesting questions of mating and ovulation cannot be answered without further study; we may conclude that both phenomena are probably quite variable as to time, that sperms are stored in the spermatheca from mating until the succeeding ovulation, and that apparently normal sperms are found for a considerable time after ovulation.

[1] This information is supplied by Dr. R. R. Humphrey. He has examined males killed in every month except April.

Development of the Spermatheca and Cloaca.

The cloaca in larvæ ranging in size from 7.7 to 10.0 cm. is very simple; there are few folds in its lateral wall and these disappear on distention of the cloaca. One, however, in the dorsal wall, persists in spite of distention. It is located just caudal to the dorso-ventral expansion into which open ureters, oviducts, gut and bladder. Because of its location, its slenderness, and its delicate epithelium, this fold is interpreted as the medial of the three folds into which the dorsal elevation divides in the adult (Figs. 14, 8, and 9). Caudal to this in the roof of the cloaca, the epithelium grows very much thicker and has a well-defined row of basal cells and basement membrane (Fig. 15).

In two larvæ of 10.3 and 10.5 cm. respectively, a noteworthy change is observable. Over a short area caudal to the slender fold described above, and dorsal to the vent, the basal row of nuclei is not distinct, and the nuclei of the epithelium are small and numerous. The overlying tissue is likewise richly cellular, and is mesenchymal in character. Upon superficial examination the nuclei of epithelium and overlying tissue are indistinguishable and seem to intermingle. Even upon closer examination they are quite similar, but a basement membrane, though very delicate, is apparent. The epithelium of this area, which stands in clear contrast to that of the rest of the cloaca, has been termed for convenience, the "cellular area," and is regarded as the anlage of the spermatheca (Fig. 16). The epithelial cells of the area show a distinct tendency to arrange themselves in rows perpendicular to the surface. Spaces often appear between the rows, but might be interpreted as artefacts. In one place a small bulb of cells has formed within the epithelium (Fig. 16*a*). Fig. 17*a* shows an early stage in the development of dorsal glands from the same animal. It will be seen that the gland tubule is in a slightly more advanced stage than the tiny bulb of cells in the cellular area. Both gland and spermathecal tubules are epithelial outgrowths.

One would probably not be justified in interpreting the picture seen in the cellular area, if taken alone, as an invasion of epithelial cells into overlying tissue were it not for the unequivocal evidence of such invasion discernible in the next stage. (The term

"invasion" is used in the embryologic sense, as a downgrowth of a bud or cord of cells of epithelial origin, not in the pathologic sense implying unrestrained growth with loss of basement membrane.)

Glands in the larva of 10.5 cm. have been referred to above. There are two groups of these; the tubules, as shown (Fig. 17 and 17*a*) are very tiny and most of them are still solid cords. The dorsal group is slightly better developed than the ventral. The former, located caudal to the cellular area, is bilateral; its tubules, numbering four to six on each side, are outgrowths from the epithelium of the roof of the cloaca, and point dorso-laterad. A ventral group of about the same number, is located cephalad to the level of the cellular area; these are outgrowths from the epithelium of the extreme ventral portion of the cloaca.

Next in the series of stages is the smallest unmistakable spermatheca, in an animal measuring 10.8 cm., captured in May; a small stub of gill left on one side gives evidence of recent transformation. It should be noted that this animal is further developed than the two previously described, although the latter were captured later in the summer (September and August respectively). This young spermatheca consists of a larger cellular area than that just described. The cells are arranged in definite rows or cords perpendicular to the surface, and most of the cords extending beyond the cellular area itself, terminate in an expanded bulb with a minute lumen. There are ten or twelve bulbs on each side, and they curve like plumes, dorso-laterad, laterad and cephalad. The less well developed tubules without definite bulbous expansions, are the most caudal of the group. The whole organ occupies less than 0.4 mm. Of especial interest is the lumen of the common tube. In its caudal portion it is pyramidal in cross-section, with the base of the pyramid dorsad (Fig. 18), and in its cephalic portion, rounded but irregular (Fig. 19). Caudally it merges into the dorsal slit; cephalicly it is replaced by the median fold which was observed in earlier stages. Both groups of glands noted previously are present. Though the ventral are but little more advanced in development, the dorsal are now small tubules with bulbous ends, similar in form to, but smaller than the spermathecal tubules. Their subsequent fate will be discussed.

It should be noted that the cellular area of the 10.3 and 10.5 cm. animals lies in the same relation to the two groups of glands and to the median dorsal fold, as does the spermatheca in the 10.8 cm. animal—namely, the ventral glands and the dorsal fold are more cephalad, and the dorsal glands are more caudad. This gives additional ground for interpreting the cellular area as the anlage of the spermatheca.

In the next stage (10.7 cm.) the whole organ has enlarged; the tubules which still spray both cephalad and laterad, are longer and more isolated from each other, and have definite lumina. Again the common tube is of interest; in its caudal portion its lumen is shaped like a short-stemmed Y, and has an epithelium three cells deep (Fig. 20); cephalad, however, the common tube is not branched, and several irregular lumina appear in the midst of the mass of cells from which the tubules spring, as though the common tube were enlarging by degeneration or pulling apart of epithelial cells as the tubules acquire lumina. Ventral glands are more numerous and open well up on the lateral walls of the cloaca. No dorsal glands are present in this animal.

A further development in an animal of approximately 11.0 cm. (tail partly broken off), is seen in the elongation and change of direction of the tubules; most of them stream conspicuously in a caudal direction and then curve ventrad (Fig. 22); others spray cephalo-laterad; while those most cephalic in position, point almost directly dorsad. In the adult there are no tubules pointing cephalo-laterad or dorsad; these have all been turned more caudad. In the 11.0 cm. animal the limbs of the Y-shaped portion of the common tube seem to be increasing in height by degeneration of epithelial cells where tubules spray out from the limbs of the Y (Fig. 23).

In the three animals just described (10.8, 10.7, and 11.0 cm.) the cloaca showed progressive increase in number and size of its folds, especially in the height and breadth of the pair lying one on each side of the median dorsal slit. These were first indicated by a slight notching in the lateral walls of the cloaca at the "cellular area" stage (Fig. 16), but developed rapidly (Figs. 18 and 20). This pair will subsequently become the caudal two

thirds of the dorsal elevation in the adult. Moreover, the median dorsal slit into which the common tube opens has deepened slightly by the development of the folds which form the elevation. As a result of this, as well as because of the further invasion of the tubules and elongation of the common tube, the organ lies farther from the dorsal wall of the cloaca in the third specimen than in the first.

Ventral glands are not altered in the 11.0 cm. animal. Of the dorsal group, three are found on each side in their old position caudal to the spermatheca. The latter are rudimentary; the height of their development was apparently reached when the spermatheca first appeared. They are exceedingly variable in all adults as was noted previously. When present, they are always located a short distance caudal to the spermatheca. The ventral group, appearing at the same time, subsequently become long, tortuous tubules with epithelium of the mucous secreting type, and are the functional glands of the adult.

The final changes in the spermatheca consist of its further removal from the cloaca, the further bending of its tubules in a caudal direction, and a change in form of the common tube. By overgrowth of the dorsal wall caudal to the spermatheca, the caudal or Y-shaped portion of the common tube is lifted farther from the cloaca, loses its direct connection with the cloaca, and communicates with it only by means of the cephalic, unbranched portion of the common tube. Meanwhile, the branches of the common tube are drawn ventrally, so that the lumen of this portion of the tube, now most distal from the opening, comes to resemble an H (Fig. 25). They are also bent caudally, hence in their most distal (caudal) part, may be seen isolated from each other (Fig. 24). The unbranched portion of the common tube (formerly its cephalic portion), now considerably enlarged, leads from the cloacal wall to the cross-bar of the H, and to the two branches. This stage (Figs. 24, 25, and 26) is an intermediate one in development, and appears at 11.2 cm., but was also seen in one full sized adult of 14.5 cm. By further expansion of the cross-bar of the H, the expanded end of the adult common tube is formed; from this large chamber its two branches extend caudally and somewhat ventrally.

The next striking changes in the cloaca are the final modification of the dorsal elevation to its adult form (cephalic portion fused, caudal portion divided), and the development of the diverticulum. The dorsal elevation, when last considered (11.0 cm.) consisted of two fairly well developed folds with the median dorsal slit between them. At the cephalic end of the slit, cephalic to the spermathecal opening, the slit was replaced by the slender median dorsal fold which has been a feature of the cloaca since early larval stages. In the 11.2 cm. animal the cellular condition of this slender fold, and its increase in breadth, seem to indicate hypertrophy. Gradually its caudal portion fuses with the two folds caudal to it; its cephalic portion remains slender, however. This fusion has been nearly completed at 13.3 cm., and fully at 13.5 cm. The hypertrophy is part of a general gradual overgrowth of the dorsal wall, which will be discussed further. By the process just described, the dorsal elevation of the adult is formed. It will be recalled that in the adult the elevation is divided in its caudal portion, but cephalic to the spermathecal opening, is fused; while still more cephalic the fused portion gives way to three folds, the median slender one, flanked by two smaller lateral ones.

The diverticulum is absent in all animals smaller than 11.2 cm.; it, likewise, is formed by the overgrowth of the dorsal wall previously mentioned. The diverticulum continues to increase in size as long as the animal grows, for in the largest animals its two prongs reach forward and partially flank the spermatheca.

Discussion.

The most interesting phenomenon in the cloaca of young *Gyrinophilus* females is the development of the spermatheca. The cellular area lying between the single mid-dorsal fold cephalicly and the dorsal glands caudally, has been interpreted in this study as its anlage. Even at this stage the mode of formation of its tubules is indicated by the arrangement of its cells perpendicular to the surface, and by a small bulb-like mass of cells apparently about to grow out into the overlying mesenchymal tissue. This invasion is very clearly shown in the next

stage. Comparison of these spermathecal tubules with gland tubules shows that both originate in the same manner.

As the tubules grow into overlying tissue, the whole cellular mass likewise sinks somewhat, and a portion of the cloacal lumen is drawn into the cellular mass to form the common tube of the spermatheca. At this stage it is not yet a tube, but rather a shallow depression, pyramidal in cross-section in its caudal part, and communicates widely with the cloaca. In this way the phylogenetic history of the common tube is repeated. One is reminded that in *Amblystoma*, the first species in the phylogenetic series to have its tubules aggregated, the common chamber is a depression in the dorsal wall, and apparently merely a modified portion of the cloaca.

Even at this early stage, however, the common tube gives evidence of enlarging by degeneration of epithelial cells, a feature which is more marked in the following stages. From the first, the caudal part of the common tube indicates its subsequent branching. The caudal portion is next lifted away from the cloaca in a dorsad direction, and becomes the distal, branched portion of the common tube, communicating with the cloaca by means of the old cephalic portion, now drawn out into a long tube.

Koehring ('25) in describing the development of the spermatheca in *Eurycea*, says: "In a 58 mm. animal . . . there are four distinct tubules. . . . These are made up of a very small group of cells closely grouped and darkly stained with barely perceptible lumina and short ducts. The ducts very nearly reach the thick walls of an invagination of the cloacal wall which is the anlage of the central tubule." She does not specifically state in her paper that she regards the spermathecal tubules as epithelial downgrowths, derived from the cloacal epithelium lining the invagination or "central tubule" (common tube); and, since she says that the ducts "very nearly reach" the walls of the invagination, one might surmise that the tubules are formed in the overlying tissue and secondarily unite with the epithelium lining the invagination. If, however, it is the lumen of the duct which very nearly reaches the lumen of the invagination, this would coincide with the findings in *Gyrino-*

philus; in this form the tubules are at first solid outgrowths, the lumen developing first in the expanded distal portion, and later in its slender neck.

The common tube of the spermatheca in *Eurycea* is regarded by Koehring (see above) as a portion of the cloacal wall. This coincides with the findings in *Gyrinophilus*. It is possible that the "thick walls of the invagination" (Koehring) correspond to the "cellular area" described in the "first unmistakable spermatheca" in this study of *Gyrinophilus*. In this specimen the mass of epithelial cells from which the tubules spring, and into which a portion of the cloacal lumen has been drawn, is very thick. It is also difficult to distinguish the basement membrane which sets off the highly cellular tissue outside from the equally highly nucleated epithelium. Together they might be said to constitute a "thick wall" for the cloacal depression or common tube.

There are several features in the development of the cloaca and spermatheca which must be accounted for. I shall consider first the change in direction of the tubules to one which is chiefly caudad. Coincident with this change, the entire spermatheca shifts from a position dorsal to the vent, to one ahead of the cephalic end of the vent, while ventral glands come to lie caudal to the level of the spermatheca. Moreover, the cloaca is relatively and actually taller in the animal of 8.8 cm. (Figs. 14 and 15) than at 10.5 cm. (Figs. 16 and 17). All these facts point toward an elongation and flattening of the cloaca, coincident with increase in length of the whole animal. In Fig. 27 the outline of an adult cloaca, relatively reduced in size, has been superimposed upon the outline of a young cloaca. The long arrows, *a* and *b*, indicate the shifting of parts which would flatten the cloaca, bring ventral glands caudal to the level of the spermatheca, and bring the spermatheca cephalic to the vent. During the change of position of the spermatheca, its tubules are turned caudad.

Elongation of the cloaca, however, does not explain the development of the dorsal elevation, the fusion of its two limbs cephalic to the spermatheca, nor the development of the diverticulum. These are interpreted as being due to an overgrowth

of the whole dorsal wall of the cloaca, as indicated in Fig. 27 by the small arrow ahead of the spermatheca, and the curved arrow behind it. This overgrowth would (1) elongate the common tube and lift its caudal portion farther from the cloaca; (2) form the dorsal elevation and cause the fusion of its two limbs ahead of the spermatheca; (3) produce the diverticulum; and (4) bring about the final change in direction of the tubules.

The two processes described above, elongation with shifting of parts, and overgrowth of the dorsal wall, are not independent but overlap in time. This is readily shown by the fact that the folds which form the caudal part of the dorsal elevation begin to form at the "cellular area" stage, and are well developed while the early spermatheca still lies over the vent.

Summary and Conclusions.

1. The cloaca of *Gyrinophilus* females is characterized by the presence of a dorsal elevation which most closely resembles that in *Eurycea*, and a diverticulum like that of the Plethodons.

2. The spermatheca is of the complex type, with a branched common tube, and numerous flask-shaped tubules.

3. Suggestive though inconclusive observations on mating and ovulation have been previously summarized (p. 264).

4. Preceded by the appearance of a cellular area, suggesting the invasion of epithelial cords into overlying tissue, the spermatheca begins to develop at the time of metamorphosis, as a mass of tubules, aggregated about a small depression in the dorsal cloacal wall which becomes the common tube.

5. Coincident with a cephalic shift in position of the whole organ, the tubules begin to change their direction to a predominantly caudal one, and the two folds forming the dorsal elevation, develop. This is followed by a fusion of these two folds cephalic to the common tube, elongation and change of form of the latter, completion of the change of direction of the tubules, and finally the development of the diverticulum. These changes in the cloaca are interpreted as being caused by cephalad growth of the dorsal wall at a more rapid rate than that of the ventral wall, and an overgrowth of the whole dorsal wall.

6. Ventral glands alone are functional in the adult, though at the time of their earliest appearance a similar group of dorsal glands appears, caudal to the anlage of the spermatheca. These undergo a limited development, and then atrophy. Remnants of the group appear in most adults.

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EXPLANATION OF FIGURES.

PLATE I.

FIGS. 1 to 10.—Diagrammatic cross-sections of the cloaca of a typical adult *Gyrinophilus* female of 16.5 cm. The levels at which these were taken are indicated on Fig. 11, a semi-diagrammatic median sagittal section of an animal of the same size. (Magnification, approximately × 11.)

FIG. 1. Section passing through vent and entrance to dorsal diverticulum.

FIG. 2. Through vent and diverticulum.

FIG. 3. Ahead of vent, through diverticulum.

FIG. 4. Ahead of vent and diverticulum; cuts through most caudal part of gland region. Though most of the glands lie slightly more cephalad, their ducts reach the surface at this level, opening upon, and between, the folds of the lateral and ventral walls of the cloaca. Dorsally, the elevation divided into two folds by the median dorsal slit, is shown.

FIG. 5. Through caudal part of spermatheca, showing the two limbs of the dorsal elevation in the dorsal wall of the cloaca; and, above, the two branches of the common tube, with tubules streaming from them.

FIG. 6. Through cephalic part of spermatheca; showing common tube opening into mid-dorsal slit.

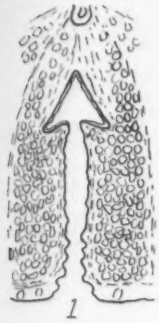
FIG. 7. Cephalic to spermatheca; showing fusion of the two limbs into a single broad dorsal elevation.

FIG. 8. Cephalic to Fig. 7; the dorsal elevation breaking into three folds, of which the median is slender and taller than the other two.

FIG. 9. Through caudal end of bladder; in dorsal wall of cloaca, the three folds seen in Fig. 8; the lateral folds are merging into the lateral wall of the cloaca.

FIG. 10. Through the dorso-ventral expansion of the cloaca. Below the cloaca communicates with the bladder. Into the dorsal expansion, farther cephalad, open the ureters and oviducts. Parts of kidneys are shown above.

FIG. 11. Semi-diagrammatic median sagittal section of cloaca of *Gyrinophilus* female of 16.5 cm. The levels of Figs. 1–10 are indicated.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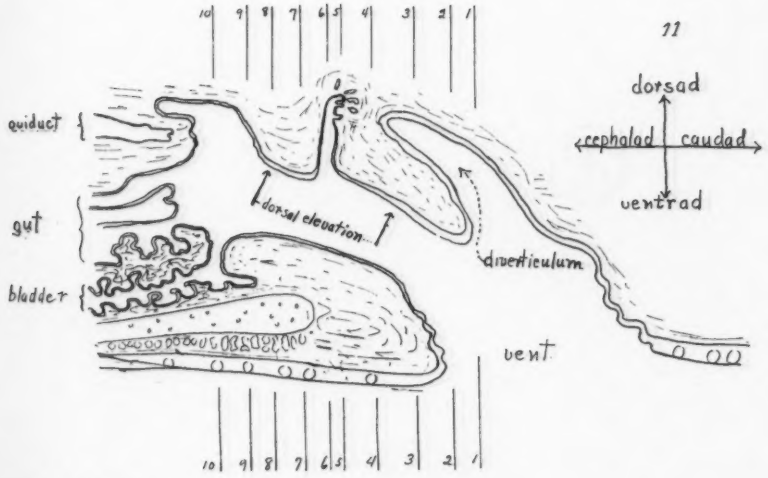

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PLATE II.

FIG. 12. Spermatheca at a level mid-way between the levels of Figs. 4 and 5, on Fig. 11. Shows the common tube at its point of junction with its two branches, tubules streaming laterally and ventrally, pigment surrounding tubules, spermatazoa in tubules. (Magnification approximately × 47.)

FIG. 13. Single tubule, showing sperms arranged in whorls. (× 97.)

FIG. 14. Cloaca of female *Gyrinophilus* larva of 8.8 cm., showing median dorsal fold. (Approximately × 33.)

FIG. 15. Same animal; more caudal level than Fig. 14; showing thicker epithelium, with well-defined row of basal nuclei and basement membrane. (× 33.)

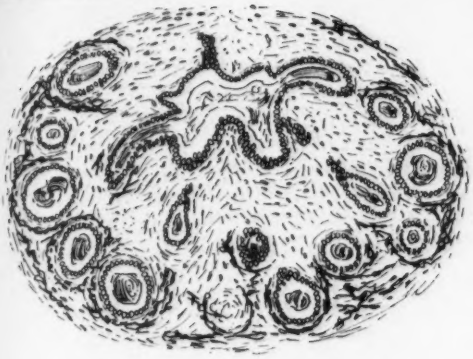
FIG. 16. Cloaca of larva of 10.5 cm., showing cellular area in dorsal wall. (× 33.) *d.e.*, anlage of one fold of the dorsal elevation.

FIG. 16*a*. Portion of cellular area under greater magnification; showing small bulb of cells, the anlage of a spermathecal tubule.

FIG. 17. Cloaca of same animal; showing anlagen of dorsal glands. (× 33.)

FIG. 17*a*. Portion of the dorsal gland area under higher magnification.

FIG. 18. Cloaca of a recently transformed *Gyrinophilus* female of 10.8 cm.; showing caudal end of developing spermatheca. (× 33.) *d.s.*, top of dorsal slit; the pyramidal area above this point is the anlage of caudal portion of common tube, at this stage not yet a tube, but a shallow depression. *d.e.*, one fold of the dorsal elevation.



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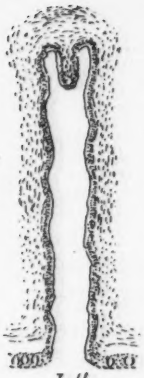

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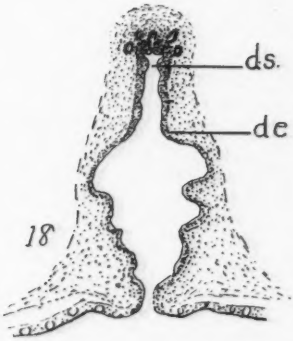

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PLATE III.

FIG. 19. From the same animal as Fig. 18, Plate II.; taken at a more cephalic level; showing spermathecal tubules growing out from the area of highly nucleated epithelium. (× 33.) *d.s.*, top of dorsal slit. *d.e.*, one fold of dorsal elevation.

FIG. 20. Dorsal elevation and spermathecal region from an animal of 10.7 cm. (× 33.) *d.s.*, top of dorsal slit. *d.e.*, one fold of dorsal elevation.

FIG. 21. Same animal as Fig. 20; taken at a more cephalic level. (× 33.)

FIG. 22. Dorsal elevation and spermatheca of animal of 11.0 cm.; showing tubules which have been turned latero-ventrad; in the center the narrow necks of tubules which terminate farther caudad. (× 33.) *d.s.*, top of dorsal slit. The cloaca is greatly distended, therefore, the two folds of the dorsal elevation are spread widely apart.

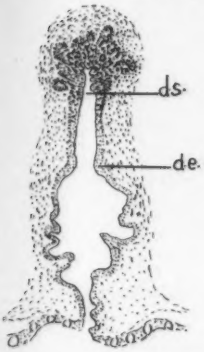
FIG. 23. From the same animal, taken at a more cephalic level; showing the Y-shaped portion of the common tube communicating with the dorsal slit; and irregular lumina forming in the cell mass above the common tube. (× 33.)

FIG. 24. Cloaca and spermatheca of an animal of 11.2 cm.; showing the two limbs of the dorsal elevation in the dorsal cloacal wall; above, the two branches of the common tube, with which tubules communicate. (× 33.)

FIG. 25. Dorsal elevation and spermatheca of the same animal, taken cephalic to the level of Fig. 24; showing the distal part of the common tube, with an H-shaped lumen. (× 33.)

FIG. 26. Dorsal elevation and spermatheca of same animal; taken at a level cephalic to that of Fig. 25; showing expanded proximal end of common tube communicating with dorsal slit. (× 33.) *d.s.*, top of dorsal slit.

FIG. 27. Diagram of median sagittal section of cloaca of young animal; in dotted lines the outline of an adult cloaca, relatively reduced in size. Arrows *a* and *b* indicate unequal growth of dorsal and ventral walls, which results in shifting spermatheca cephalic to vent and turning tubules caudad. Small arrows indicate overgrowth of dorsal wall, which elongates common tube, and produces dorsal elevation and diverticulum.



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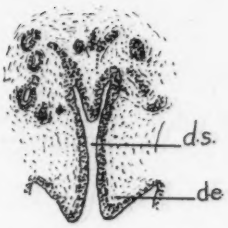

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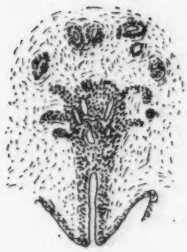

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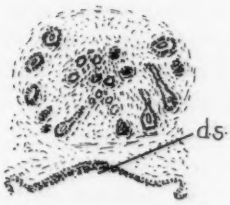

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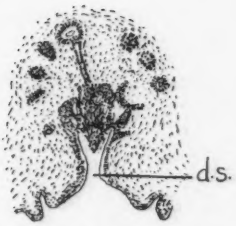

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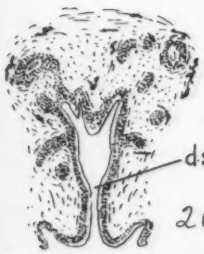

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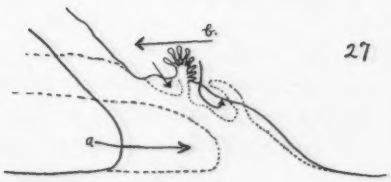

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THE CLOACA AND SPERMATHECA OF *HEMIDACTYLIUM SCUTATUM.*

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Two specimens of adult female *Hemidactylium scutatum* were examined, one measuring 7.1 and the other 7.7 cm., both captured in August.[1] After fixation in Bouin's fluid, the cloacae were sectioned, one transversely and one sagittally, at 12 μ and 10 μ respectively. The ova of both animals were less than 1.0 mm. in diameter, since ovulation in this species occurs during May (Bishop, '18).

The cloaca of *Hemidactylium*, when empty, is slit-like, with its lateral walls thrown into quite regular folds. Two such folds, located in the dorsal wall of the cloaca, and first discernible at the level of the cephalic end of the vent, become fairly tall as one passes cephalad. These two together constitute the dorsal elevation, which though varying in form, appears in the cloaca of the female in several urodeles (Kingsbury, '95). The slit between the two folds or limbs of the dorsal elevation is continuous caudally with the roof of the cloaca over the region of the vent, and cephalad, with a dorsal expansion of the cloaca, into which, as in other forms, the ureters and oviducts open. In other words, the two limbs of the elevation are not fused in their cephalic portion as they are in adult *Gyrinophilus*, but remain separate throughout their extent, as in developmental stages of the latter. A single median dorsal fold, partially dividing the dorsal expansion, recalls the similar slender fold ahead of and continuous with the fused portion of the elevation in *Gyrinophilus*.

The oviducts have large lumina and folded walls, as is to be expected from the relatively large size of the ova in this form. The bladder opens into the cloaca on its ventral side, somewhat cephalic to the level of the oviducts. There is no dorsal diver-

[1] I am indebted to Dr. R. R. Humphrey for the material used in this study.

ticulum (recess), a feature which appears, among the forms so far described, only in the Plethodons and *Gyrinophilus*.

The sperm-storing organ is confined to the region above the dorsal elevation. It consists of a few tubules (three on one side and four on the other in one of the animals), which open into the lateral walls of the dorsal slit in its extreme cephalic portion, just at the point where it passes over into the dorsal expansion. From these openings, the long slender necks of the tubules extend caudally; each neck terminates in an irregularly branched end-piece. Branching of spermathecal tubules, observed in only a few instances in the series of *Gyrinophilus*, is conspicuous in the end-pieces of the tubules of *Hemidactylium*. Moreover, some of the terminal branches in this species are not bulbous, but longer and more slender, resembling large tubular glands. The region in

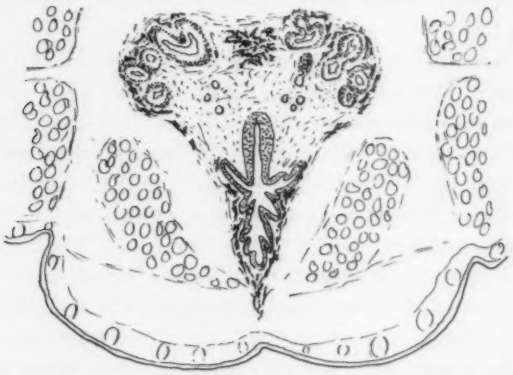

FIG. 1. Cloaca of *Hemidactylium female* (adult, 7.1 cm.), at a level some distance caudal to the openings of the spermathecal tubules. In the dorsal wall of the cloaca, the two folds comprising the dorsal elevation are seen, with the median dorsal slit between them. Just above, on each side, are the narrow necks of spermathecal tubules, which open separately into the dorsal slit cephalad to this level; above these, the expanded, branched end-pieces of spermathecal tubules. Pigment is seen between the two groups of tubules, around the organ as a whole, and in the wall of the cloaca. (× 30.)

which the spermathecal tubules open is free from glands, but opening into the more caudal portion of the dorsal slit, intermingled with the caudal ends of the spermathecal tubules, there is a group of seven or eight pairs of very small, slightly tortuous gland tubules.

The glands described here are all confined to the region of (*i.e.*, located in the substance of) the dorsal elevation, but there are in addition two other groups. One group, consisting of four or five tubules on each side, lying dorsal to and opening into the roof of the cloaca, is located entirely caudal to the elevation. This group is separated by a short but definite interval from those intermingled with the spermathecal tubules. The other group, also of four or five pairs, is located in the region of the dorsal expansion, therefore cephalic to the spermatheca.[2]

FIG. 2. Cloaca of *Hemidactylium* (adult, 7.1 cm.) at a level through the openings of the spermathecal tubules into the lateral walls of the mid-dorsal slit. Comparison with Fig. 1 shows that, as the tubules pass cephalicly toward their openings, they also slope ventrally, and now lie within the substance of the dorsal elevation. The elevation is composed of two folds or limbs with the mid-dorsal slit between them. The elevation ends abruptly a few sections cephalic to this level. Above, the mid-dorsal slit is already merging into the dorsal expansion ahead of the elevation. The tubules on the right side end one section cephalic to this level; on the left side, two of the three tubules are seen bending sharply toward the slit. (The difference between the sides is due to slight obliquity of sectioning.) Pigment appears above the cloaca. (× 30.)

The epithelium of these glands is emphatically not that of functional gland tubules, as seen in the ventral group in adult *Gyrinophilus;* for the tubules are minute, the lumen barely discernible in most of the tubules, and the cytoplasm in each cell scanty. They closely resemble early stages in the development of glands in young Gyrinophilus females, and are apparently rudimentary or only slightly developed structures. Rudimentary dorsal glands were found in *Eurycea* (*Spelerpes*) in two groups (Kingsbury '95), one cephalic to and one caudal to the opening of the sperma-

[2] Another adult measuring 5.5 cm. (with ova 1.0 mm. in August), which has since been examined, shows only the most cephalic of these three groups of glands. They are probably quite variable, as are the rudimentary glands of *Gyrinophilus*.

theca, and in *Gyrinophilus* in one group, caudal to the spermatheca. *Eurycea* and *Gyrinophilus* however, have functional ventral glands, while *Hemidactylium* has none, in this respect resembling the Plethodons and *Desmognathus*.

Bishop states that mating in *Hemidactylium* probably takes place in the spring; he bases this conclusion on the fact that males and females may be found together beneath logs or stones at this time of the year, and on the observation that the ova in the ovaries of animals in the succeeding month, were all less than 0.5 mm. in diameter. The latter fact, to my mind, indicates only that ovulation has recently occurred, and proves nothing as to the time of mating. In the present study, the spermathecal tubules of one of the animals (7.7 cm.) killed in August, are filled with sperms. These are arranged in whorls, as has been noted in other species when sperms are abundant. The animal would seem to have mated after its May ovulation (ova in the ovary are less than 1.0 mm. in size). This finding is in harmony with the idea that sperms may be stored in the spermatheca of urodeles for a considerable time, and that the organ is a device for insuring fertilization of ova in animals which lead a solitary life for a large part of the year.

Gyrinophilus and the seven forms described by Kingsbury, fall rather naturally into a phylogenetic series, as was discussed in the previous paper. The trend during phylogeny seems to have been toward the aggregation of the tubules about a specially modified portion of the cloaca [3] the reduction in number of tubules and loss of their flask shape, and the suppression of glands. *Hemidactylium* cannot be placed in the series, which begins with *Amblystoma*, and which is characterized by the modification of a portion of the cloaca into a common tube, for it has retained the primitive plan of spermatheca seen in *Necturus;* namely, tubules opening separately on the lateral walls of a mid-dorsal slit. It typifies nevertheless, two other tendencies noted in the phylogenetic series; namely, reduction in number and change in form of spermathecal tubules, and reduction in number and functional activity of glands.

[3] Kingsbury's suggestion that the depression into which spermathecal tubules open in *Amblystoma*, and the common tube of the organ in more highly developed forms, are really a portion of the cloaca, seems justified by the manner of development of the spermatheca in *Gyrinophilus*, and in *Eurycea* (Koehring, '25).

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THE STRUCTURE AND TRANSFORMATION OF THE LABIUM OF *ANAX JUNIUS*.

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INTRODUCTION.

During a recent investigation of the degenerative changes in the muscles of transforming Odonata the author found it convenient to use the retraction of the labium as an index to approaching internal changes. For some hours before the final ecdysis the insect remains quiet, without feeding and partly out of water. It is during this time that the retraction of the labium becomes evident. Some attention was consequently given to its condition and structure. When the literature of the subject was searched it yielded little upon structure, beyond that of the chitinous skeleton, and nothing regarding changes during transformation, in spite of the fact that the larval labium has long been cited in both popular and technical treatments as one of the most uniquely adaptive structures in insects. To quote Amans, "Cette piece a de tout temps été jugée comme très curieuse et très intéressante; tous l'ont décrite avec une minutieuse complaisance, et Dufour en profite pour faire intervenir la Providence et les causes finales."

Miss Butler's paper (2) is quite universally cited as the authoritative treatment of the Odonate labium. It is based upon extensive comparative studies of the configuration of the labium in all of the subfamilies, and her bibliography includes the works of many of the greater insect anatomists as far back as 1842. Rather unaccountably, however, she omits Amans' paper on the larva of *Aeschna grandis* (1), and states that "The powerful muscles and hinges which constitute the mechanism of the labium have never hitherto been investigated" (p. 112). This is followed by a very brief and apparently incorrect description of the mechanism. Her interpretations are in turn followed by Tillyard (4) in 1917.

Amans (1) studied the larval labium with considerable care but he, also, overlooked some of the most evident and important

structures, and was consequently led astray regarding most of the major functions, *i.e.*, extension and flexion of the labium and the abduction of the lateral lobes. It may further be stated that he applied no modern system of nomenclature to the muscles and other structures described.

Neither of these authors published adequate figures of the larval structures. All available illustrations of the imaginal labium are concerned solely with the skeletal parts, and the transitional stage has never been figured.

For these reasons an investigation of the structure of the labium of the larva and of the adult and its transitional condition has seemed worth while.

Materials and Methods.

The specimens of *Anax junius* studied were fixed in Bouin's fluid or injected with 95 per cent. alcohol. Both fluids gave good results, though the yellowing of the muscles by the picric acid made it possible to clear and mount dissections without resorting to further staining. In fact, the definition and relations of the muscles in such preparations was often superior to those stained in borax carmine. Specimens were fixed in both the extended and flexed positions.

While a part of the work was done from cleared and mounted specimens, details of the structure were obtained much more efficiently by the dissection of alcoholic materials under a Greenough binocular microscope. By this method the parts could be pinned down in 70 per cent. alcohol in a translucent paraffin tray for dissection with needles, and viewed alternately by reflected and transmitted light. Care was taken to clear away all fat or coagulated body fluids which might interfere with clear vision. Adult labia were cleared and studied in oil without mounting.

Most of the drawings were outlined under the camera lucida, though a few were made to measurement.

The Labium of the Larva.

General Structure.

The labium of a full grown larva is between sixteen and twenty millimeters long when extended (Fig. 1). It consists of two main

divisions, the submentum and the mentum. The latter is somewhat the longer of the two, and is roughly spatulate in shape; the former is subcylindrical.

The mentum terminates apically in a rounded, median lobe which bears a median notch and is edged with short bristles. On either side of the median lobe is a lateral lobe bearing a moveable hook and an end hook. These possess no muscles. Each lateral lobe is hinged near the outer border of its base, while the medial lobe is prolonged into a long triangular tendon which lies in a deep slit-like invagination in the anterior edge of the mentum and receives the insertion of the adductor muscle. Proximally the mentum narrows to a trifle more than half its apical width, and deepens to accomodate the heavy adductor and abductor muscles. It is hinged to the submentum as shown in the various figures, the carinae being heavily chitinized.

The submentum is flattened on the face opposed to the mentum, otherwise it is nearly cylindrical. Its distal end is rounded and projects beyond the hinge at which the mentum is received. It is within this rounded, elbow-like projection (menton, of Amans) that many of the important muscle attachments occur. The submentum is hinged closely to the ventral side of the head but possesses such freedom of movement as to enable it to swing instantly from its normal position between the first and second pairs of legs, when the labium is folded, to one in nearly the same plane beneath and in front of the head. It is supported by the posterior branches of the tentorium and surrounding skeletal parts. A large outward fold between the head and base of the submentum, supported by a T-shaped chitinous rod running forward and downward to the base of the hypo-pharynx (ancre, of Amans), aids in giving this freedom for the forwards thrust (Fig. 1). Little lateral movement is possible at this hinge and practically none at that between the submentum and mentum. The submentum accommodates two pairs each of extensor and flexor muscles.

Larval Musculature.

Muscles Activating the Lateral Lobes.—The lateral lobes are moved by two pairs of strong muscles, the abductors and the adductors.

The abductors of the lateral lobes lie wholly within the mentum (Figs. 1, 10 and 11). They are strong flattened muscles lying laterally, broad at the origin upon the dorsal surface of the skeleton slightly distad from the hinge and tapering to a point at the insertion of the tendon upon the outer side of the lateral lobe, where a slight prominence gives leverage. The mesial side of each abductor overlaps the corresponding adductor muscle. A tracheal branch lies ventral to it laterad of the adductor.

The adductors of the lateral lobes are the largest muscles of the labium (Figs. 1, 8 to 13). The origin is upon the central area of the rounded extension of the submentum where it gains excellent support from the heavily chitinized and concave surface. The muscle tapers to its insertion upon the long tendon attached to the mesial side of the lateral lobe. As shown in Fig. 8 this muscle seems to be comprised of a dorsal and a ventral layer. In Fig. 9 most of the muscle had been dissected off. In the region of the hinge and about their origins the adductors are flanked by the secondary (accessory) flexors (Fig. 13). Fig. 9 shows how the adductors may also aid greatly in flexing the labium. The first effect of their contraction being the adduction of the lateral lobes, that of continued contraction across the hinge, flexion.

Muscles Activating the Mentum and the Submentum.—Four pairs of muscles within the labium are concerned with its extension and flexion, the primary flexors, the secondary (accessory) flexors, the primary extensors, and the secondary (accessory) extensors.

The primary flexor muscles lie in the ventral portion of the submentum (when the labium is folded). They have their origins side by side upon a V-shaped tongue of chitin on the median ventral region of the base of the submentum (Fig. 1). They run distally, accompanied by a pair of tracheal trunks, taper gradually, pass over the hinge lateral to the adductors, and insert upon the ventral surface of the mentum at its proximal end. Each is attached to a short, transverse, chitinized, thickening projecting mesiad from the lateral carina (Figs. 1, 9, 10 and 12). In passing around the adductor muscle it narrows to nearly tendon-like proportions. A comparison of Figs. 8, 9, and 10 will make clear its action as a flexor.

The secondary flexor muscles are located in the region of the

hinge. They have not been described previously. In the contracted condition they are somewhat fan-shaped (Figs. 9 and 11). The origins of the muscles lie at their broad ends, the attachments to the submentum nearly circling those of the adductors (Fig. 13). From here they pass ventrally next to the skeleton to insert for a very short distance on the ventro-lateral floor of the mentum just proxinad to the origins of the abductors of the lateral lobes. When the labium is extended they assume so different a form that they have probably been mistaken by earlier workers for the proximal ends of the abductors. The dissection shown in Fig. 10 indicates the true relations. It will be noted that each passes laterad to the chitinous process, which serves for the insertion of the primary extensor muscle.

The primary extensor muscles occupy the dorsal portion, when the labium is folded, of the submentum (Figs. 1 and 8 to 13). Their origins are quite far apart upon the ends of the posterior branches of the tentorium. They are heavy at the bases but each soon tapers to attach to a small cord-like tendon which passes between the adductor of the lateral lobe and the secondary flexor to a triangular chitinous process projecting from the mentum into the cavity of the rounded elbow of the submentum. This mechanism is a very nice adaptation for increasing the leverage of this strong extensor muscle. A comparison of Figs. 9 and 10 leaves no doubt of the mode by which the extensor functions.

The secondary (accessory) extensor muscles have also escaped description in the past. They are small band-like muscles attached to the meso-lateral regions of the elbow of the submentum (Figs. 5, 6, and 8 to 10). Their origins are at the extreme distal end of the submentum; their insertions latero-ventral to those of the secondary flexors. Their function seems to be that of producing in the skeleton of the elbow of the submentum the necessary increased curvature during extension of the labium.

The Labium during Transformation.

As indicated earlier, a period precedes transformation during which the larva ceases to feed and wanders restlessly in and out of the water, finally remaining quietly on a twig or other support

with the thorax in the air. It seems to be during this restless period that the labium is remodeled and retracted into the submental portion of the old skeleton, though it has been impossible to observe the steps in this process. It is accomplished in a few hours at most, perhaps in a much shorter time, as there appears little evidence of profound histological change. Practically all of the stages collected were similar to those illustrated in Figs. 2 and 3. The mentum and lateral lobes of the larval skeleton are entirely empty and the labium has retracted into the basal two thirds of the old submentum. Its parts are straightened out and rolled laterally to fit the tube-like case, while the whole labium stands out ventrally from the under side of the head. When viewed under low magnification most of the parts of the adult labium are visible but they are thicker and more fleshy in appearance and are closely rolled together. The broad lateral and median lobes are especially conspicuous. Attempts to flatten out the excised organ are not usually successful as the tissues are very delicate and the parts tear badly. However, a median longitudinal section dividing the labium into halves, made possible the determination of the following facts.

As all of the internal structures are so crowded together it is difficult to make out their relations. However, the larger muscles are quite clear as to identity. The primary flexor muscles are very long and may be followed laterally around the bases of the adductors as in the larva. Each is accompanied by a large, degenerating trachea. The adductors have contracted to form very short, thick muscles. They are curved ventrally at their origins and insert as in the larva. They can be identified with assurance by their insertion and relations to the primary flexors. The remaining muscles cannot be so definitely followed but may be seen in general outline, tending towards vertical or slightly oblique positions in the mentum and submentum. As these latter are so rolled up, however, it is probable that the muscles might assume diverging positions as in the adult, if the labium was flattened out.

In the ventral, distal portion of the mentum are two rounded masses of some size which suggest degenerating muscles. Comparison of the adult with the larva indicates the loss of the ab-

ductors and it is possible that these two masses represent them at this stage. No histological examination has as yet been made.

The tracheæ which are to remain in the adult are seen wound vertically up and down between the main muscles. The T-shaped chitinous support mentioned in connection with the larval labium is very conspicuous, and bears a large flattened tooth-like ventral process.

The Labium of the Adult.

General Structure.

The labium of the adult is much shorter, thinner, and more expanded than that of the larva. When flattened out it measures approximately five millimeters in length by eight millimeters in diameter, including the lateral lobes. That is, it is less than a third as long as, and only a third wider than, the larval structure from which it arose. The added width results from the expansion of the lateral lobes. The latter with the greatly enlarged median lobe, form the mask which covers the mouthparts. In their normal positions the lateral lobes and the median lobe rise almost vertically from the borders of the floor-like mentum-submentum to enclose the sides and front of the mouth; the whole structure is nearly cup-like.

The function of the labium is changed to a considerable extent. In the larva it is a remarkable organ of prehension, in the imago it has become mainly one for the retention of prey caught in the basket of the spiny legs. Its moveable hooks now resemble palps possessed of a single small terminal tooth or spine each. While formidable in size and appearance the end hooks are weakly joined to the lobes (Fig. 4). They would be of little use without the pressure and support applied to their outer faces by the flexing of the median lobe (Fig. 7), converting them into a sort of fork to hold the food in the mouth.

The mentum is relatively short and very wide due to the formation of a pair of squames laterally by the development of longitudinal folds and chitinous ridges along the areas of muscle attachment. The hinge between it and the submentum is less flexible than in the larva, but possesses a distinct enlargement along its median half. Most of the muscle attachments are grouped on

either side of this enlarged portion. The submentum is greatly reduced but offers support for the origin of all of the larger muscles (Figs. 4 to 7). The unique adaptations for extension and flexion so marked in the larva are gone.

Adult Musculature.

There are five pairs of muscles in the imaginal labium. These are greatly reduced in size and lack much of the precision of adjustment of the earlier condition. A comparison of the larval, transitional, and adult structures has led to the following homology. There is nothing to indicate that more than one pair has been destroyed. Many are clearly identified by their attachments and relations, while a few others have been difficult to determine except by elimination and by evident function. They may be located on Figs. 4 to 7.

Muscles Activating the Lateral Lobes.—The abductors of the lateral lobes. These muscles are missing in the imago. The two rounded masses of apparently degenerating muscular tissue in the transitional labium may represent them. Abduction of the lateral lobes is now accomplished indirectly by the pull of the secondary flexors on the squames.

The adductors of the lateral lobes. This pair of muscles is clearly in its larval position. The origin of each is upon the ventral wall of the submentum just proximal to the hinge or angle. The area of attachment is circular or oval in form, and is close to the median line. The insertion is, as in the larva, upon a strong tendon at the inner angle of the lateral lobe. This inner angle is distinctly offset dorsally (*i.e.*, towards the buccal cavity) so that a contraction of the muscle produces flexion of both the lateral lobe and the median lobe as well as adduction of the former. In shape it is subcylindrical and tapers from its origin to its insertion in a half spindle. It lies mesiad of other muscles and somewhat parallel to the primary flexor.

Muscles Activating the Mentum and Submentum.—The primary extensor muscles are the longest in the adult labium. They are of depressed spindle shape, and lie in the median line between the bases of all the diverging muscles. In dissecting the labium free

from the head they are often torn loose at their insertions and withdrawn. The origins are upon a chitinous semi-circle near the base of the submentum; the insertions on the dorsal surface (*i.e.*, towards the buccal cavity) of the extreme proximal region of the mentum, one immediately on either side of a slight median carina-like thickening.

The secondary (accessory) extensor muscles. These small muscles lie transversely on the floor of the apex of the submentum, one on either side ventral to the middle region of the primary and secondary flexors. They are of a depressed cone shape, about 40° in diameter at the origin. The origin is upon a chitinous semi-circle projecting from the ventral wall of the distal end of the submentum midway between the median line and the lateral margin. The insertion is upon a short tendon from the extreme proximo-lateral angle of the squame. Without careful focusing with a 16 mm. objective this seems on the lateral margin of the submentum; practically, the pull is upon both at once.

The primary flexor muscles are probably the strongest pair in the labium. They are heavy and cylindrical, and lie between the primary extensors and the adductors medially and the secondary flexors laterally, dorsal to the transverse secondary extensors. They originate on semi-circles at the base of the submentum with their inner sides close together. The insertion of each is on a fairly large circular area of the ventral surface of the mentum at its proximo-lateral angle (exclusive of the squame), and marked on the surface by a convexity lateral to the thickened central portion of the hinge.

The secondary flexor muscles are thin and band-like, and twisted at an angle of about 90° about the lateral aspects of the primary flexors. They are crossed midway and ventrally, as indicated above, by the secondary extensors. The origin is upon a transverse chitinous semi-circle located ventro-laterally about midway of the length of the submentum. The insertion is upon a similar semi-circle on the latero-dorsal aspect of the squame.

Summary.

Studies carried out upon the larval, transitional, and adult stages of development show that:

1. But one pair of muscles breaks down (the abductors of the lateral lobes) during transformation. The remainder are reduced in size and changed in their attachments only in so far as the new functional conditions necessitate.

2. Changes in the function of certain pairs is clear, as in the secondary flexors which take on the duty of abduction in the imago.

3. Two pairs of muscles are here described for the first time: the secondary extensor, and the secondary flexor muscles. New details regarding the mechanism of extension and flexion are also described.

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DESCRIPTION OF FIGURES.

All figures are of the labia of *Anax junius*. The drawings were made through the camera lucida or to measurement by the author. Dotted lines indicate the forms of parts which underlie others and which could usually not be seen without further dissection or the clearing of the specimen. The magnifications are expressed in diameters.

adl —adductor muscle of the lateral lobe.
abl —abductor muscle of the lateral lobe.
bc —buccal cavity, mouth parts removed.
ca —carina.
exp —primary extensor muscle.
eh —end hook.
exs —secondary or accessory extensor muscle.
fxp —primary flexor muscle.
fxs —secondary or accessory flexor muscle.
hb —hind border of the head.
hy —hypo-pharynx.
hd —head, sectioned.
kr —chitinous T-shaped rod.

lal —lateral lobe.
m —mentum.
ml —median lobe.
mh —movable hook of lateral lobe.
mx —base of maxilla.
sd —opening of salivary duct.
skl —skeleton of larval labium, severed at hinge between the mentum and submentum.
sm —submentum.
sq —squame of mentum.
tn —central plate of tentorium.
tnp —posterior branch of tentorium.
tr —trachea.

PLATE I.

Fig. 1. Extended labium of the larva, viewed from below and showing the muscles and tendons as seen through the transparent skeleton. × 6.

FIG. 2. Retracted labium of the larva as it appears a short time before the insect transforms. Ventral view of head and dorsal view of the labium. The hypo-pharynx, bases of the maxillæ and other structures are shown surrounded by the loose larval skeleton. × 9.

FIG. 3. A ventral view of the same labium shown in Fig. 2. × 9.

FIG. 4. Extended and flattened labium of the adult in ventral view showing the muscles. × 9.

FIG. 5. Ventral view of the mentum of the adult. The labium in this and the next two figures is in normal condition, not flattened out. The muscles and their attachments are drawn in one half only in order to show the skeletal features on the opposite side. × 9.

FIG. 6. Ventral view of the submentum of the specimen shown in Fig. 5. Other conditions the same. × 9.

FIG. 7. A view into the cup-like labium of the adult from the dorsal aspect. The muscle relations are shown, and in addition the mechanism of the end hooks and the median lobe. × 9.

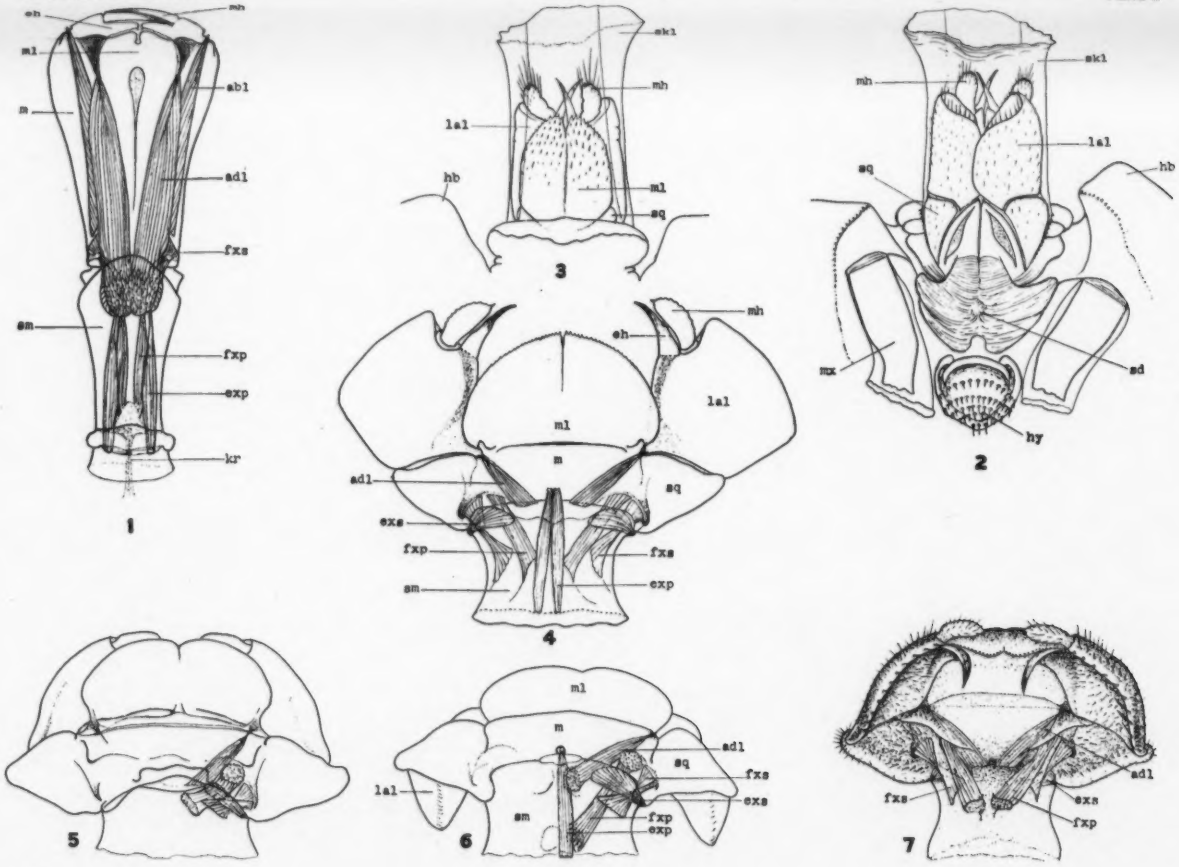

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PLATE II.

FIG. 8. Right half of the head and flexed labium of the larva as viewed from the inner aspect. Parts of the tracheal branches which lie between the adductor muscles are omitted. × 9.

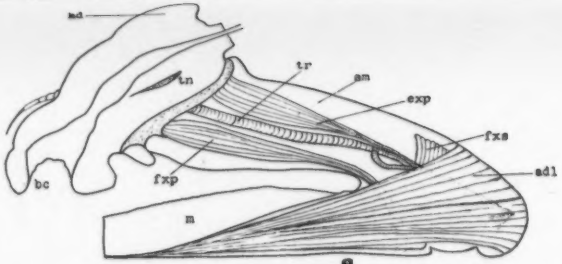
FIG. 9. Left half of the labium shown in Fig. 8, so placed as to illustrate, together with Fig. 8, the complete labium opened like a book. In Fig. 9 the adductor muscle has been dissected away to give a complete view of the mechanism for extension and flexion. Chitinized processes are stippled. × 9.

FIG. 10. Left half of the extended larval labium as seen from the interior. A large portion of the base of the adductor muscle is dissected away to uncover the extensor and flexor muscles. The primary and secondary flexors should be especially noted. × 9.

FIG. 11. The flexed larval labium viewed from the exterior right side, the muscles showing through the transparent skeleton. × 9.

FIG. 12. Ventral view of the mentum and the main hinge of the larval labium when flexed. All internal parts seen through the skeleton. × 9.

FIG. 13. Dorsal view (with labium flexed, ventral when extended) of the submentum of the specimen shown in Fig. 12, illustrating the attachments of muscles. × 9.



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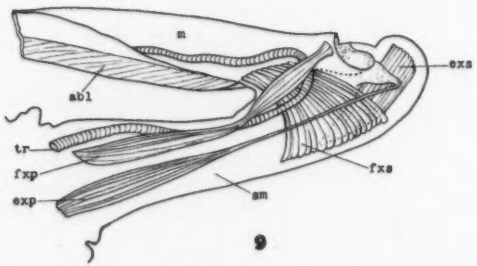

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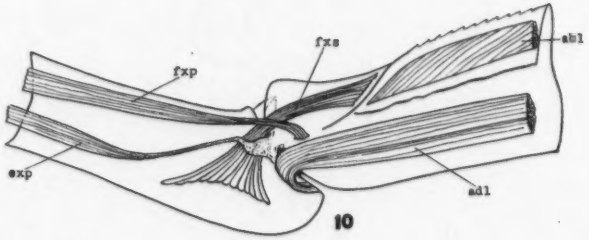

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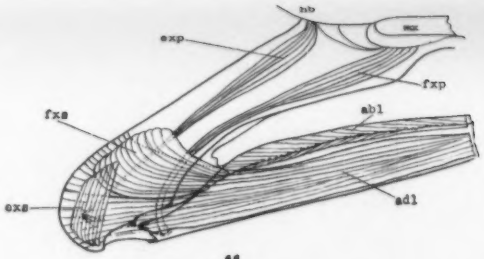

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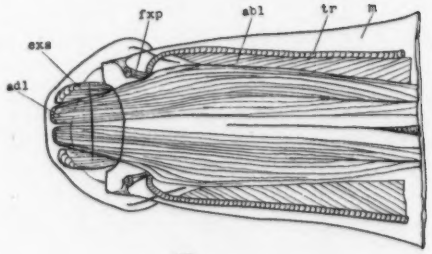

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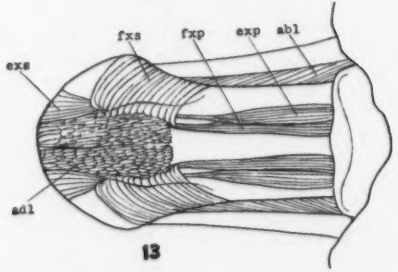

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